INTERACTIONS OF *PUCCINIA HORDEI* AND *ERYSIPHE GRAMINIS* ON BARLEY.

by

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Abstract.

Using techniques designed to provide uniform and reproducable infections of P.hordei and E.graminis on first seedling leaves of Zephyr barley, it was shown that these pathogens developed somewhat differently when in combination. Under greenhouse conditions P.hordei developed less well on leaves previously inoculated with E.graminis and similarly E.graminis developed less well on previously rusted leaves. P.hordei appeared to be most vunerable in the early stages of its infection, leading to reduced numbers of pustules as well as reduced size of pustules on mildewed leaves. The establishment of E.graminis appeared to be unaltered by previously inoculating leaves with P.hordei but the development of colonies and the production of conidia were considerably reduced on pre-inoculated leaves. Both of these effects were greater in situations where the levels of the first disease were higher and where the period between the inoculations were longer, they were not so great where specific fungicides were used to eradicate the 'first' pathogen or where plants were treated with materials likely to increase the carbohydrate level within the leaves.

In situations where leaves were inoculated with P.hordei and E.graminis within the same 24h they often appeared to become more susceptible to P.hordei, this was however, a somewhat variable effect.

In field trials carried out in 1974 and 1975 where rust and mildew were allowed to develop in combination and, by the use of specific fungicides to develop separately on three cultivars, it was found that the levels of rust were significantly higher on plants where mildew was controlled than on plants where both diseases developed. Mildew however was affected little by the presence of rust.

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Contents

Introduction

In situations where barley is grown it often becomes infected with Erysiphe graminis, causal fungus of powdery mildew, and Puccinia hordei, causal fungus of brown rust. Much is known about the effects of mildew and a fair amount (though relatively less) about brown rust. Little is known however about the effects which these two pathogens have in combination and how they interact. A preliminary investigation by Simkin, (1973) indicated that the presence of one of these pathogens could affect subsequently the development of the other.

Interest in these aspects is further stimulated by the fact that mildew and, indeed, brown rust can be controlled by specific fungicides and the question arises as to whether the control of one pathogen could lead to an increase in the other.

The present investigation aimed to examine the interactions between these two pathogens in detail, to characterize them; to evaluate the factors which influence these interactions most and to determine the nature of the effects.

Literature Review

Johnson and Huffman(1958) reported a marked local antagonism between Puccinia coronata, causing crown rust of oats and P.recondita the fungus causing leaf rust of wheat. On wheat susceptible to leaf rust which was inoculated with P.coronata fewer pustules of P.recondita developed and these were of a different infection type to those produced on plants not so pre-inoculated. The pre-inoculation of wheat with P.coronata caused chlorotic flecks, a resistance reaction which presumably triggered the resistance to P.recondita. Similarly, Kochman & Brown(1975) found that when oat leaves were pre-inoculated with either P.graminis tritici or P.recondita tritici fewer pustules of the compatible rusts P.graminis avenae and P.coronata avenae developed from subsequent inoculations than on control plants. Ersek(1973) reported that the number of mildew colonies from inoculations with E.graminis f.sp.tritici were reduced by pre-inoculating wheat with the barley form of E.graminis. This reduction was associated with a defence reaction by the plant to the first inoculation. On plants similarly 'immunized' and then inoculated with race 11 of P.graminis tritici, the number of rust pustules that developed was only 10% of the number on control plants. He concluded that the effect was a general phenomenon, which developed locally in pre-inoculated leaf parts. Yarwood,(1956) reported similar effects on Puccinia helianthi on sunflower leaves pre-inoculated with Uromyces phaseoli, and Littlefield,(1968) found that infection of flax by Melamspora lini was reduced by pre-inoculating the leaves with either an avirulent

strain of the same fungus or with wheat rusts such as P.graminis tritici and P.recondita tritici.

Relatively few investigations, however, have been concerned with interactions between virulent obligate parasites. The reports that there are indicate two different situations. Firstly, the presence of one pathogen increases the amount or severity of the second. Thus Johnston,(1934) reported that wheat leaves became more susceptible to rust (P.triticina) when they were previously infected with mildew. Similarly Manners and Gandy,(1954) showed that there was an increase in susceptibility of wheat leaves to rust (P.triticina) around mildew colonies, but that with heavy mildew infections the rust was almost completely inhibited. Also Van der Wal,Shearer and Zadoks(1970) reported that more severe symptoms developed from inoculations with Septoria nodorum on wheat already infected with P.recondita. In this instance, the production of uredospores was inhibited and the formation of teleutospores was induced.

Secondly, the presence of one pathogen decreases the amount or severity of the second. In greenhouse experiments using Zephyr barley inoculated with P.hordei(race Pb 60/3/1) and E.graminis(race or races unknown) Simkin,(1973) found that the presence of one pathogen on the leaf reduced the level of the subsequently inoculated 'second' pathogen. He also found that the closer in time the two pathogens were inoculated onto the same leaf the more marked was the reduction in establishment of both pathogens.

The nature of these effects are complex and remain speculative. Obligate parasites have a highly specific nutrient requirement (Bose & Shaw, 1960) and the susceptibility of their hosts is often affected in many ways. Floating leaves on solutions thought likely

to alter their metabolite balance (Samborski & Forsyth, 1960); altering the membrane permeability(Thatcher,1942); or altering the levels of free amino acids and carbohydrates (Lyles, Futrell & Atkin,1949) are a few examples.

Interest in interactions is however not entirely academic, since these could be important in the field and provide natural limitation of some pathogens, for example the control of mildew on barley might result in more brown rust. Simkin and Wheeler,(1974b) and Yarnham, Bacon and Hayward,(1971) did not detect increases in brown rust on field grown barley where mildew was controlled. The rust levels in the field trials carried out by Simkin were very low and the disease did not develop until fairly late in the season, and the assessments of brown rust made by Yarnham et al.1971 were on winter barley early in the 'rust' season (early June). Brooks,(1972) reported that ethirimol seed dressing(specific mildew fungicide) significantly reduced brown rust attack, again using winter barley, but also reported a slight increase in brown rust on three out of fourteen spring barley trials where mildew was controlled. In fungicide trials at the Plant Breeding Institute Johnson, Scott and Wolfe,(1970) reported that brown rust was more severe on all plots where mildew was controlled. This had the effect of making the determinations of yield losses due to mildew inaccurate. A similar epidemic of brown rust in 1971 (Wolfe & Minchin,1971) was also reported to be more severe on plots where mildew was controlled. However there is little conclusive evidence for increases in brown rust due to the control of mildew, or indeed, for the suppression of rust by the presence of mildew.

MATERIALS AND METHODS

Production of plant material.

Most experiments were performed on the first seedling leaf of the barley cultivar Zephyr. Mercury-dressed seed (Elsom's of Spalding Ltd.) was sown, c. 2cm. deep, in John Innes Potting Compost No. 2 in 13cm. plastic pots which were then kept in filtered-air cabinets designed to exclude fungal spores (Finney, 1973). These cabinets were built on a self-watering sand bench within a greenhouse, maintained at 18±3°C and with a 16h. photoperiod. Under these conditions seedlings developed a fully expanded first leaf in 10 days. Seedlings with abnormal or misshapen first leaves were rogued, where necessary, to give a uniform stand, usually between five and ten seedlings per pot.

Fungal material.

(a) Erysiphe graminis. Collections of E.graminis were maintained on seedlings of Zephyr barley; kept in different filtered-air cabinets to maintain their identity. There were two collections: one used mainly in preliminary experiments, was a field isolate (race or races not determined) from Silwood Park, obtained in 1972, the other used in later experiments, was an isolate of race B 72/27 obtained from the Plant Breeding Institute, Cambridge.

(b) Puccinia hordei. One isolate was used throughout and was derived from the collection of race (Pb. 60/3/1, 295/21) obtained by Simkin & Wheeler (1974a). Further uredospore collections were obtained by infecting seedlings of the barley cultivar Deba Abed (Milstem-dressed) and then removing the uredospores daily from pustule eruption to

leaf senescence with a cyclone collector. These spores were either stored at 1°C or were freeze-dried. Under these two conditions spores remained viable for 3 - 4 months and 12 months respectively.

Inoculation of plants.

Considerable difficulty was experienced in preliminary experiments in obtaining uniform inoculations of plants with P.hordei and especially with E.graminis so the apparatus shown in Fig. 1 was designed to ensure a more uniform deposition of spores on the seedling leaves.

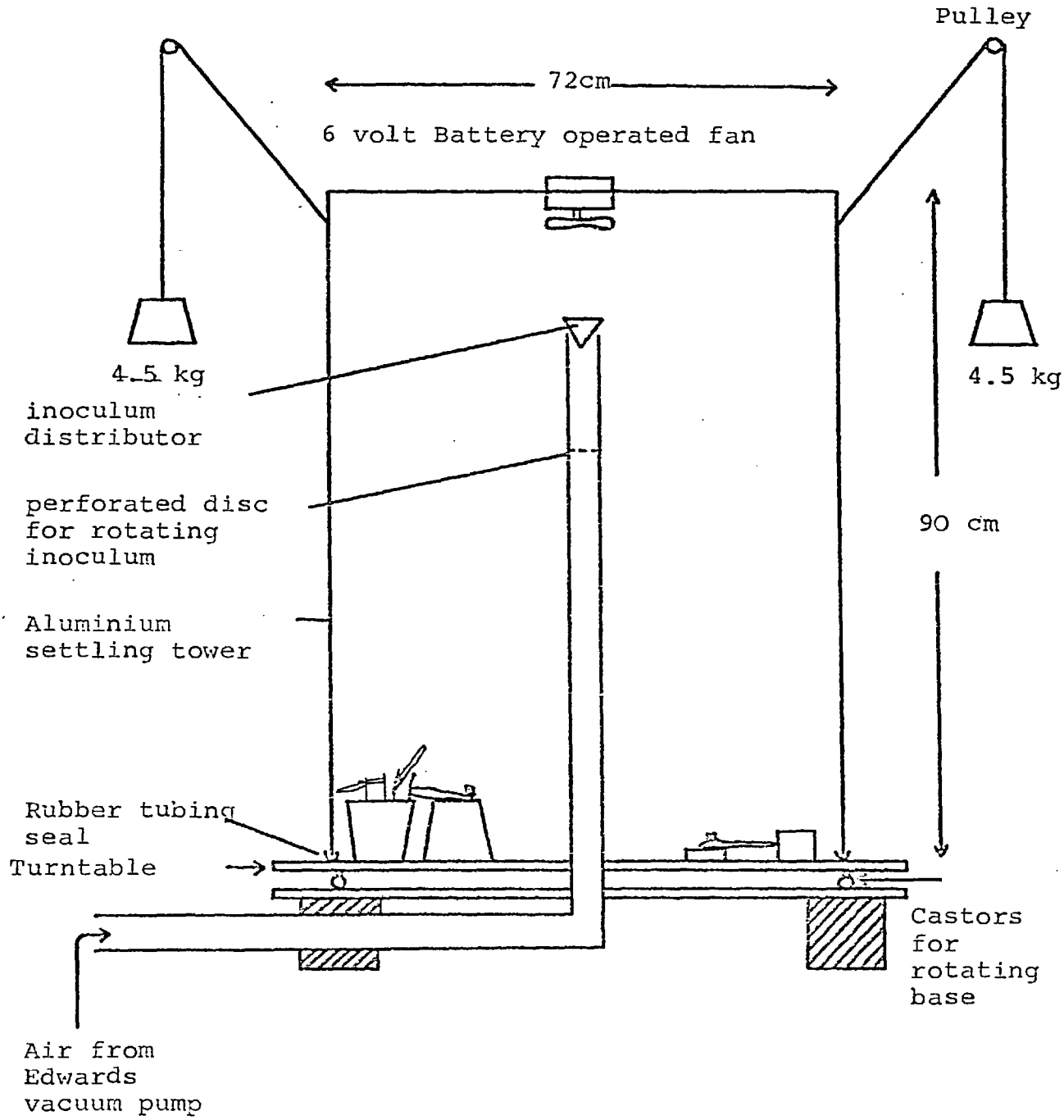
It consisted basically of an aluminium settling tower, 0.7m. diameter and 1m. high, suspended by a system of pulleys and counterbalances over a wooden turntable of similar diameter on four furniture castors. The top of the settling tower was sealed with a perspex lid into which, centrally, was incorporated a 6v battery fan unit. The bottom rim of the tower was cushioned by a length of split rubber tubing so that the turntable could still rotate when the tower was lowered.

A perspex tube, inner diameter 25mm., was positioned so that it arose centrally through the turntable to a height of 650cm. above it. The upper 250cm. of this tube (inoculum carrier) was detachable and had at its base a plate perforated with small holes, cut diagonally and arranged in a spiral and at its top a 25mm. plastic funnel weighted with putty. The lower part of the perspex tube was connected via a T-piece to an Edwards pump.

For inoculations the apparatus was operated as follows:

(a) The aluminium settling tower was raised and pots of seedlings were placed at random on the turntable. The leaves were held

Fig.1 Inoculation chamber.



horizontal by gently running a finger downwards from the tip to the base of the abaxial surface. For inoculations with P.hordei (but not those with E.graminis) they were also sprayed with water using either an ASL Spraymist or Shandon Chromatography Sprayer.

(b) Inoculum was placed on the perforated disc of the carrier. This consisted either of a weighed amount of a uredospore/talc mixture, for inoculations with P.hordei or several mildewed leaves, for inoculations with E.graminis (see Appendix 1). The funnel and inoculum carrier were then replaced and the settling tower lowered carefully into position over the plants.

(c) Both the Edwards pump and the battery fan were switched on. Air blown into the perspex tube by the Edwards pump lifted and spiralled the inoculum in the carrier and also lifted the funnel allowing spores to discharge into the settling tower. The battery -operated fan unit further mixed these spores with air at the top of the tower.

(d) After 30 seconds the pump and fan unit were turned off simultaneously and the suspended inoculum allowed to settle for 15 - 30 mins. During this period the turntable was revolved slowly by hand.

Plants inoculated with E.graminis were transferred without further treatment to filtered-air cabinets. Pots of plants inoculated with P.hordei were stood in warm water (20 - 25 C) contained in a galvanised tray, 67 x 97cm., and covered with polyethylene on a wooden frame (56 x 88 x 65cm.). The inside of this polyethylene cover was also sprayed with water. After 24 hours in this humid chamber these plants were also placed in filtered-air cabinets.

Assessment of disease.

Under the conditions described above rust pustules erupted 8 - 9 days after inoculation and mildew colonies were visible by day 6. Rust pustules were usually counted 10 days after inoculation and mildew colonies 7 days after inoculation. Some rust infections grew through the leaf producing pustules on both surfaces. To avoid duplicate counts the leaf was held up to the light when assessing pustules by eye or if the leaf was detached this was placed over a light box.

In some instances growth of the fungi was measured. Leaves were detached and cleared in methanol for 24 hours. They were then stained in lactophenol cotton-blue either for 10 - 15 minutes (for mildew colonies) or for several hours (for rust pustules). Colony area was determined from measurements of length and breadth using a calibrated graticule in the eyepiece of the microscope.

Spore germination.

Germination of rust uredospores and mildew conidia used as inocula were checked by tests carried out in chambers as described by Manners & Hossain (1963). Germination of spores on inoculated leaves was examined by pressing the leaf firmly on to a piece of Sellotape. The spores remained attached to the Sellotape when the leaf was peeled off and were examined after staining in lactophenol cotton-blue.

EXPERIMENTAL.

1(a). Preliminary Investigation.

Simkin (1973) found that there was an interaction between Puccinia hordei and Erysiphe graminis on the first seedling leaf of the barley cultivar Zephyr.

The preliminary experiments described in this first section were designed to provide more evidence of these interactions and to characterize them fully.

The two situations: the development of P.hordei on plants preinoculated with E.graminis and the development of E.graminis on the plants preinoculated with P.hordei were both investigated.

Development of E.graminis on the first seedling leaves of Zephyr barley, previously inoculated with P.hordei.

Effects of different amounts of P.hordei and different periods between the two inoculations.

Simkin (1973) used high levels of rust in his experiments so it was felt necessary to establish that similar effects on E.graminis could be obtained using smaller inocula of P.hordei.

Uredospores of P.hordei were weighed out and diluted one to one with talc. After mixing thoroughly half of this preparation was mixed with an equal weight of talc. This procedure was repeated to give the different inocula shown in table 1.

Two pots of uniform, 10-day old Zephyr barley seedlings were treated with each inoculum and seedlings in another two pots inoculated with talc only as a control. The inoculation procedure was basically similar to that of Simkin (1973). The pots of seedlings were placed at the base of a settling tower (1m x 6m), sprayed with a fine mist of water, and the inoculum dispensed into the air at the top of the tower. After allowing the spore/talc mixture to settle, the plants were resprayed with water and kept in a saturated atmosphere for 24 hours (Simkin & Wheeler 1974). They were then transferred to the cabinets in which they were grown and after a further 3 days all plants were inoculated with E.graminis. Again the inoculation procedure was as described by Simkin (1973). The pots of plants were arranged at random around a central cardboard cylinder (58 x 13cm) which was mounted 2·6 cm. above the sand bench and housed in its base a small electric fan. Apparatus and plants were sited within a filtered-air cabinet. Inoculum was introduced into the top of the cylinder, the fan was turned on for 3 minutes and then suspended conidia allowed to settle onto the plants for 30 minutes.

All plants were subsequently kept under normal greenhouse conditions until they were assessed for mildew after 7 days.

Although in this experiment there were marked effects on mildew development of pre-inoculating plants with P.hordei the amounts of mildew and rust were respectively too high and too low, so a second experiment was set up. This combined the treatments of the first experiment with a study of the effects of different periods between the two inoculations.

Table 1 Effects on mildew development of pre-inoculating leaves with different inocula of P.hordei.

Inoculum level	Uredospore/ talc mixture†	Mean no. rust†† pustules per leaf	Total mildew colonies per 20 leaves
0 (control)	talc only	0	169
1	1 : 1	300 - 350	27
2	1 : 3	250 - 300	2
3	1 : 7	200 - 300	4
4	1 : 15	100 - 250	9
5	1 : 31	100 - 200	10

† 0.2g. per two pots of seedlings

†† Visual estimates

Standard error ± 0.6446
(mildew colonies)

Uredospores of P.hordei were mixed with talc to give a 1 : 3 dilution (inoculum level 2 of Table 1) and a range of inocula more dilute than those of the previous experiment (inoculum levels 5, 6, 7 and 8 of Table 2). Two pots of uniform 10 - day old seedlings were treated with each inoculum and four similar pots of seedlings were inoculated with talc only to serve as controls for both parts of the experiment. Subsequently two pots of seedlings were inoculated with the 1 : 3 uredospore/talc mixture 6h or 1, 2, 3, 4 days later to give treatments A to E of Table 2. All plants were placed in a saturated atmosphere for 24h immediately after inoculation except those in treatment A which had only 6h. in these conditions. Four days from the start of the experiment all plants were inoculated with E.graminis and then returned to the greenhouse bench. Mildew was assessed 7 days and rust 10 days from inoculation.

The results (Table 2) show that inoculating leaves with P.hordei 4 days before inoculation with E.graminis markedly reduced the number of mildew colonies that developed even when the rust level was as low as 25 - 75 pustules/leaf. The magnitude of the reduction appeared to be increased with greater rust levels (Table 2(A)) and with longer periods between inoculations (Table 2(B)).

Rust and mildew levels were variable, and not always uniformly distributed about the leaf, and it was observed that an important feature in the interaction was the pattern of the disease distribution.

Table 2 Effects on mildew development of pre-inoculating leaves with P.hordei.

(a) Different inocula of P.hordei, inoculation 4 days before that with E.graminis.

Inoculum level	Uredospore/ talc mixture†	Mean no. rust†† pustules per leaf	Mean no. mildew colonies per leaf
0	talc only	0	25.7
5	1 : 31	150 - 200	3.7
6	1 : 63	100 - 150	2.6
7	1 : 127	75 - 100	17.1
8	1 : 253	25 - 75	14.5

(b) Different periods between inoculations: inoculation with a 1 : 3 uredospore/talc mixture†

Treatment	Days between inoculations	Mean no. rust pustules per leaf††	Mean no. mildew colonies per leaf
A	0.25	100 - 200	16.5
B	1	200 - 250	9.4
C	2	250 - 300	6.6
D	3	250 - 300	3.0
E	4	250 - 300	7.2

† 0.2g. applied per two pots of seedlings

†† Visual estimates

Standard error ± 0.7457

Development of P.hordei on the first seedling leaves of Zephyr barley, previously inoculated with E.graminis.

Effects of different periods between the two inoculations.

In these preliminary experiments no attempt was made to use a range of mildew levels.

Fifteen pots of barley seedlings were grown in a spore - free cabinet for 10 days. The seedlings in ten pots were inoculated with E.graminis using the method described earlier in this section. Five of these pots were returned to the greenhouse bench and mildew allowed to develop (mildew control of Table 3). Subsequently one pot of seedlings inoculated with E.graminis and one pot of untreated seedlings were inoculated with P.hordei to give periods between inoculation with E.graminis and P.hordei of 2h or 1, 2, 3, 4 days and the corresponding 'rust controls' (Table 3). The numbers of mildew colonies and rust pustules per leaf were recorded for each group of plants 7 and 10 days after the appropriate inoculations. The results indicated that when leaves were inoculated with E.graminis 4 days before inoculation with P.hordei the number of rust pustules that developed was significantly lower than in the corresponding control. With intervals of less than 4 days between the two inoculations, results were variable and otherwise only with a day interval were significantly fewer rust pustules formed on mildewed plants.

TABLE 3 (See also Appendix Table 3) — Effects on rust development of preinoculating leaves with E.graminis.

Time between Inoculations.	Mean no. pustules or colonies per leaf. 'Rust Control' leaves		pre-inoculated with E.graminis. Rust	Mildew	'mildew control'
2 h.	330	(N.S.)†	418	95	158
1 day	264	(*)	160	196	-
2 days	213	(N.S.)	254	182	-
3 days	400	(N.S.)	466	125	-
4 days	191	(***)	12	160	-

† Significant differences between amounts of rust on mildewed and rust control leaves at $P \leq 0.05$ and $P \leq 0.001$ indicated by * and *** respectively. Based on t-test.

Rust and mildew on other leaves and other cultivars.

Although variable results were obtained from the above experiments, they served to show that on the first seedling leaf of Zephyr barley, a reduction in mildew could be obtained by a prior inoculation with P.hordei, and that in some instances, rust could be reduced by pre-inoculating leaves with E.graminis. Obviously, both situations required further investigation. However, it seemed necessary to establish first that the effects observed were not peculiar to the first seedling leaf of Zephyr barley, so some experiments of a similar type were made on the second leaf of Zephyr seedlings and on seedling leaves of other cultivars. These are described here.

Effects of dual inoculations with E.graminis and P.hordei: on second seedling leaf of Zephyr barley.

Forty-eight pots of Zephyr barley, grown in John Innes No. 2 potting compost, were kept in a filtered-air cabinet until the seedlings had a fully-expanded second leaf. These were then divided into three groups (A,B,C) each of sixteen pots. The seedlings in group A were inoculated with E.graminis, those in group B with P.hordei and those in group C not inoculated.

Immediately (time 0) and 2, 4, 6 days later two pots were taken at random from group A and from group C and the seedlings were inoculated with P.hordei. Similarly, at these times, two pots were taken at random from group B and from group C and these seedlings were inoculated with E.graminis. All plants were incubated in

comparable conditions. Amounts of mildew and rust were recorded 7 and 10 days respectively after inoculation on both the first and second leaves. In some instances, mildew was so extensive that only % leaf cover could be estimated but usually the numbers of mildew colonies and rust pustules per leaf were recorded. Data relating to the first leaf are given in Appendix Tables 4 & 5 and those for the second leaf are summarized in Table 4.

The results clearly indicate that with the exception of day 0 pre-inoculating leaves with one fungus markedly reduced the growth of the second fungus, the magnitude of this reduction being directly related to the period between inoculations with the two fungi.

TABLE 4 (See also Appendix Tables 4-7) — Effects of dual inoculations with E.graminis and P.hordei: second seedling leaf of Zephyr barley.

Days between Inoculations.	Mean no. pustules or colonies per leaf.			
	Rust development on leaves		Mildew development on leaves	
	Pre-inoculated with E.graminis	Not pre-inoculated	Pre-inoculated with P.hordei	Not pre-inoculated
0	257 - NS -	228	(70%)† - .002 -	(90%)†
2	74 -.001 -	274	40 - .001 -	70
4	15 - .02 -	38	28 - .001 -	68
6	15 -.001 -	122	73 - .001 -	165

† Colonies confluent so an accurate count was not possible.

* Statistical significance based on t-tests. (Values for P as shown in the Table)

Effects of dual inoculations with E.graminis and P.hordei: On different leaves of Zephyr barley.

Under field conditions in the South of England and in the absence of any fungicide treatment, most plantings of barley become infected to some extent with E.graminis and P.hordei. Mildew often develops on the lower leaves early in the growing season, brown rust develops later and usually affects only the last-formed leaves of the barley plants.

The following experiment investigated the effect which mildew on the first three seedling leaves had on the development of rust on leaves 4 and 5.

Barley seedlings in ten pots were grown in a filtered-air cabinet until three leaves had fully developed. The seedlings in five pots were then inoculated with E.graminis by placing them at the base of a settling tower and shaking infected plants over them. Fourteen days later, when the leaves 4 and 5 had fully expanded the seedlings in all ten pots were inoculated with P.hordei by spraying them with uredospores suspended in 10^{-4} tween 80 using a Shandon Chromatography Sprayer. The plants were incubated and the pustules on leaves 4 and 5 counted 10 days later.

Although pre-inoculation of leaves 1, 2 and 3 with E.graminis appeared to reduce the amount of rust developing on leaves 4 and 5 (Table 5) the plant to plant variation was so great that these differences were not significant.

TABLE 5 (See Appendix Table 8) Effects of dual inoculations with E.graminis and P.hordei: on different leaves of Zephyr barley.

Replicate	Mean no. rust pustules/leaf: Seedlings pre-inoculated (leaves 1,2,3) with E.graminis.		Seedlings not pre-inoculated (control)	
leaf no.	4	5	4	5
1	33*	20*	38	17
2	18	32	22	44
3	8	31	31	61
4	2	43	5	36
5	3	7	7	17
Mean	10.5	19.3	23.9	38.0

* Not significantly different from controls.

Effects of dual inoculations with E.graminis and P.hordei: Mildew development on the first seedling leaves of different cultivars pre-inoculated with P.hordei.

Seedlings of the five barley cultivars (Sultan, Berac, Midas, Julia and Zephyr) were grown in a filtered-air cabinet, three pots per cultivar for 10 days. The seedlings in two pots of each cultivar were then inoculated with P.hordei. Four days later the seedlings in all pots were inoculated with E.graminis. The number of mildew colonies on each leaf was counted 7 days after inoculation. On each cultivar mildew development was significantly less than on the corresponding control (Table 6).

The reverse situation, where the development of rust on leaves of different cultivars pre-inoculated with E.graminis is examined in a later section.

TABLE 6 (see also Appendix Table 9.) Effects on mildew development of pre-inoculating leaves of different cultivars with P.hordei

	Mean no. colonies per leaf on				
Leaves	Sultan	Berac	Midas	Julia	Zephyr
pre-inoculated with P.hordei	16.7†	4.4†	2.5†	11.0†	8.5†
Leaves not pre-inoculated (controls)	79.3	60.5	27.6	37.6	89.8

†Significantly different from the controls at $P \leq 0.001$

Effects of dual inoculations with E.graminis and P.hordei: Experiment using improved inoculation techniques.

While the above experiments indicated that pre-inoculating leaves with one pathogen reduced the development of a second pathogen and that this was not an effect confined solely to the first seedling leaf of Zephyr barley, they also indicated the need for better methods of inoculating plants with E.graminis and P.hordei so that the results would be less variable. This led to the development and testing (Appendix 1) of the techniques described on Page 11 .

Also, since there were so many possible combinations of disease levels (i.e. as a result of using different inocula) and periods between inoculations, it was decided in future experiments to examine interactions with two disease levels, designated 'high' and 'low' with inocula of each pathogen applied to plants either immediately after each other (Time 0) or after an interval of 2, 4 or 6 days. In these instances, a 'high' disease level refers to the development of between sixty and 120 rust pustules or mildew colonies per leaf following inoculation with P.hordei or with E.graminis only and, correspondingly, a 'low' disease level refers to the development of less than forty pustules or colonies per leaf. To achieve these levels the following inocula were placed in the 'inoculum carrier' of the apparatus shown in Fig.1 : for 'low' mildew, ten first leaves of seedlings each with 60% - 80% cover of mildew (race B72/27 of E.graminis) and shaken vigorously 48hours before required that a new crop of conidia would be available for inoculation; for 'high' mildew, twenty such leaves; for 'low' rust 5 mg. and for 'high' rust 15 mg. of rust uredospores (race

derived from collection Pb 60/3/1, 295/21) diluted to 1 g. with talc. Standard methods of inoculation and incubation were used throughout (P 11) and mildew colonies and rust pustules were counted 7 days and 10 days after the appropriate inoculations.

Using these techniques a series of experiments were performed to cover the different treatment-combinationslisted in Table 7 , plus appropriate controls, and to give data suitable for multiple regression analysis. These data are detailed in Appendix Tables 10 and 11. The results of earlier experiments were also included in analyses where appropriate.

Two multiple regressions were performed. Table 8 summarises the analysis of data for the disease situations in which leaves were inoculated with P.hordei before inoculating them with E.graminis The dependant variable is the amount of mildew, as a percentage of its appropriate control, with the period between inoculations, rust levels and mildew levels on (P.hordei) treated plants as independant variables.

The analysis showed that the most important factor in reducing mildew development on leaves pre-inoculated with P.hordei was the number of successful rust infections. Length of the period between inoculations was also important and was negatively correlated with the dependant variable. The positive correlation between amount of mildew and the dependant variable (i.e.mildew as % of control) also indicated that 'high' levels of mildew were reduced proportionally less than 'low' levels.

Table 9 summarizes the analysis of data for the reverse situation in which leaves were inoculated with E.graminis before

TREATMENT COMBINATIONS

TABLE 7

	First Inoculation	Second Inoculation.	
(1)†	High rust	High mildew	(5)
(1)	High rust	Low mildew	(6)
(2)	Low rust	High mildew	(5)
(2)	Low rust	Low mildew	(6)
(3)	High mildew	High rust	(7)
(3)	High mildew	Low rust	(8)
(4)	Low mildew	High rust	(7)
(4)	Low mildew	Low rust	(8)

† Numbers indicate the different inoculations .

Entries in the table with the same number indicate that plants were treated with the same inoculum.

Table 8 (see also Appendix Table 10) Effects on mildew development of pre-inoculating leaves with P.hordei. Multiple Regression Analysis.

Variables	Mean	Standard deviation	Correlation X Y	Regression coefficient	S.E. of Regression coefficient	Computed 't' value	Probability (P)
X 1	2.95	1.98	-0.334	-4.167	1.32	3.16	0.01
X 2	92.07	91.30	-0.704	-0.196	0.03	6.40	0.01
X 3	38.05	41.26	0.623	0.264	0.07	3.80	0.01
Y	58.67	31.89					

Analysis of variance

Source	D.F.	Sum of squares	Mean square	F value	Probability.
Regression	3	36002.96	12000.99	36.29	0.01
Residual	48	15872.48	330.68		
Total	51	51875.44			

Variables:

X 1 = Period between inoculations

X 2 = Mean no. rust pustules per leaf on dual inoculated leaves.

X 3 = Mean no. mildew colonies per leaf on dual inoculated leaves.

Y = Percentage of the appropriate control of mildew on dual inoculated leaves.

Table 9 (see also Appendix Table 11) Effects on rust development of pre-inoculating leaves with E.graminis.

Multiple Regression Analysis.

Variables	Mean	Standard deviation	Correlation X Y	Regression coefficient	S.E. of Regression coefficient	Computed 't' value	Probability (P)
X 1	2.46	2.04	-0.640	-16.167	3.85	4.20	0.01
X 2	93.44	70.06	-0.450	-0.330	0.11	3.06	0.01
X 3	71.385	113.56	0.291	0.103	0.07	1.52	n.s.
Y	84.03	64.01					

Analysis of Variance.

Source	D.F.	Sum of squares	Mean square	'F' value	Probability
Regression	3	84866.99	28288.9	13.981	0.01
Residual	35	70819.99	2023.4		
Total	38	155686.97			

Variables:

X 1 = Period between inoculations

X 2 = Mean no. mildew colonies per leaf on dual inoculated leaves.

X 3 = Mean no. rust pustules per leaf on dual inoculated leaves.

Y = Percentage of the appropriate control of rust on dual inoculated leaves.

inoculating them with P.hordei. The dependent variable here is the amount of rust as a percentage of the appropriate control. The length of the period between inoculations appeared most important together with the number of successful infections with E.graminis.

Dual infection of rust and mildew: Effects of pre-inoculating leaves with E.graminis.

The effects of dual inoculations of E.graminis and P.hordei which were indicated by the preliminary experiments were examined in more detail. This section deals specifically with experiments in which an inoculation with E.graminis preceded that with P.hordei.

1. Effects in different parts of inoculated leaves.

Nine pots, each containing five, 10-day-old Zephyr barley which had been grown in a filtered-air cabinet were used. The seedlings in six pots were inoculated with E.graminis. Three days later, the seedlings in three of these pots plus those not yet inoculated were inoculated with P.hordei. All seedlings were kept in a saturated atmosphere for 24h before they were returned to a filtered air cabinet. Ten days after the inoculation with P.hordei the leaves were cut from all plants. The extreme tip and base of each leaf was discarded and the remainder was cut into three, 3cm portions. The width of both ends of each segment was measured, its area calculated using the standard formula for a trapezium and the numbers of mildew colonies and rust pustules counted on each segment.

The results (Table 10) showed that the development of rust was little affected by the levels of E.graminis used in this experiment. However, it was interesting that the development of mildew was itself reduced on leaves inoculated with P.hordei and this effect was most pronounced in the distal part of the leaves.

TABLE 10 (See also Appendix Table 13) Dual infections of rust and mildew: Effects in different parts of the leaf of pre-inoculation with E.graminis.

	Mean no./cm^2 of.†			
	Mildew Colonies.		Rust Pustules.	
Leaf Section / Inoculum:-	E.graminis only	E.graminis + P.hordei	P.hordei only	
Base	3.81	-ns- 4.09	11.93	-ns- 11.29
Middle	3.64	-ns- 2.97	34.29	-ns- 29.40
Tip	4.67	3.44**	56.62	-ns- 48.22

† based on a sample of 15 leaves per treatment.

** Significantly different ($P \leq 0.01$) from corresponding control.

Inoculation of leaves with E.graminis, followed immediately by inoculation with P.hordei.

In some experiments where an inoculation of leaves with E.graminis was immediately followed by an inoculation with P.hordei, the resulting number of rust pustules per leaf was higher than on leaves inoculated with P.hordei only. This apparent increase in susceptibility was, in most instances, quite small but was of interest especially in view of the report by Manners & Gandy (1954) that the susceptibility of wheat to brown rust was increased in the vicinity of mildew colonies. Some of the results of Simkin (1973) also indicated a similar effect in dual infections of E.graminis and P.hordei.

The following experiment sought more information on this point.

Ten pots of uniform, 10-day old Zephyr seedlings were prepared. The seedlings in five pots were inoculated with E.graminis then immediately all pots were inoculated with P.hordei. All pots were incubated in a saturated atmosphere for 24h. and then returned to the greenhouse bench. Nine days later, the leaves were detached and cleared in methanol for 48h. The number of pustules on each leaf was counted by placing the cleared leaves over a light box and the number per unit leaf area calculated using measurements of individual leaf area. The mean number of rust pustules per cm^2 on mildewed leaves was 47.3 compared with a mean of 37.5 for control leaves, a difference significant at $P = 0.001$ in a 't' test (Appendix Table 12).

2. Effects of inoculating different surfaces of a leaf.

Nine pots of 10-day-old Zephyr barley were prepared. The leaves of seedlings in three pots were secured in the inoculation chamber with their adaxial surfaces uppermost and similarly seedlings in three pots were arranged with their abaxial surfaces uppermost. All the leaves were then inoculated with E.graminis. After 4 days, mildew could just be distinguished on the inoculated leaf surface. Then all inoculated leaves together with leaves of seedlings in the three pots not yet treated were fixed with their adaxial surfaces uppermost and were inoculated with P.hordei. All plants were incubated in a saturated atmosphere for 24 h. Immediately following this five leaves were sampled from each treatment : leaf impressions of their upper surfaces were taken using Sellotape. The remaining plants were returned to the greenhouse for a further 9 days when the first leaves were removed and cleared in methanol for 24 h.

Mean% germination of rust uredospores was determined from fifty microscope fields selected at random on each leaf impression. The number of rust pustules per leaf was counted and their sizes determined from measurements of length and breadth.

Percentage germination of rust uredospores was similar in all treatments (Table 11) but significantly fewer and smaller pustules developed on upper surfaces already inoculated with E.graminis.

TABLE 11 (See also Appendix Table 14) Dual infections of rust and mildew: Effects on rust development of pre-inoculating different leaf surfaces with E.graminis.

	Rust development of upper leaf surface.		
	Mean % germination of uredospores.	Mean no. of pustules/leaf.	Mean area (mm^2) of pustules.
Inoculations with:			
P.hordei only (control)	34.9	116.0	0.194
E.graminis, abaxial surface; P.hordei adaxial surface.	34.2	116.5	0.175
E.graminis & P.hordei on adaxial surface.	30.3	35.8*	0.149**

Values significantly different from control at $P \leq 0.05$ and $P \leq 0.01$ denoted by * and ** respectively.

3. Effects on the development of rust.

This experiment was designed to determine the stage in development at which rust was affected by pre-inoculating leaves with E.graminis. The information from the multiple regression analysis (p 30) indicated a maximum effect on rust development when leaves were inoculated with large amounts of E.graminis 6 days earlier, so these conditions were used in the following experiment.

Six pots of Zephyr seedlings were used. The adaxial surfaces of the first leaves in three pots were inoculated with E.graminis. After 6 days, when the inoculated leaves already bore abundant mildew, all seedlings (including those not yet inoculated) were inoculated with P.hordei. After incubating the plants in a saturated atmosphere for 24 h, five leaves from each treatment were removed and impressions of their upper surfaces taken with Sellotape. All plants were then placed in the greenhouse. After a further 48h, five more leaves were sampled from each treatment, these were cleared in methanol for 24h. The numbers of rust pustules were counted on the remaining leaves 10 days after inoculation.

Percentage germination of uredospores was determined from the leaf impressions as in the previous experiment. The cleared leaves were stained and examined microscopically to determine the fate of the uredospore germ tubes but this proved not to be feasible because germ-tubes were masked by hyphae of E.graminis. Scanning electron microscopy of the same material also failed to resolve this problem. As an alternative the number of primary rust infections was

recorded in each treatment from thirty fields taken at random from the central portion of each leaf (Table 12).

Table 12 (See also Appendix Table 15) Dual infections of rust and mildew: effects on rust development of inoculating leaves with large amounts of E.graminis 6 days earlier.

	Rust development on upper leaf surface.		
	Mean % germination of uredospores.	No. primary infections†	Mean no. pustules/leaf.
Inoculated with:			
P.hordei only	50.2	64	57
E.graminis and P.hordei	51.7	3**	2.2**

**Statistically different from controls at $P \leq 0.01$.

† Totals for 150 fields.

Dual infections of rust and mildew: effects of pre-inoculating leaves with P.hordei.

1. Effects of inoculating different parts of the leaf with P.hordei.

Six pots of 10-day-old Zephyr barley were used. The first leaves of the seedlings in one pot were inoculated by dusting a uredospore/talc mixture onto the lowest (basal) portion with a camel-hair brush so that a third of the adaxial surface was treated. The leaves of seedlings in a second pot were similarly treated so that a central portion was inoculated and those in a third pot so that the distal portion was inoculated, all on the adaxial surface. The plants in the remaining three pots were inoculated with talc only to give controls corresponding to the three treatments with rust uredospores. All plants were incubated in a saturated atmosphere for 24h (18 - 23°C) and then 3 days later they were inoculated with E.graminis. The number of mildew colonies per leaf was recorded after a further 7 days under greenhouse conditions.

The results (Table 13) showed that mildew development was most affected by inoculating the basal third of the leaf with P.hordei.

TABLE 13 (See also Appendix Table 16) — Dual infections of rust and mildew: effects on mildew of pre-inoculating different parts of leaves with P.hordei.

Portion of leaf pre-inoculated with P.hordei.	Mean no. mildew colonies per leaf.
Basal third	39.4*
Central third	74.0
Distal third	71.1
Nil (control).	83.2

* Significantly different from the control ($P \leq 0.05$).

2. Effect of three different inoculum levels of P.hordei on mildew development.

Twelve pots of 10-day-old Zephyr barley were used. The seedlings in nine pots were inoculated with P.hordei using 5mg uredospores to 1g talc. Three pots were then removed from the inoculation chamber and the seedlings in the remaining six pots re-inoculated with P.hordei using 10mg uredospores to 1g talc. Another three pots of seedlings were removed and seedlings in the remaining three pots inoculated a third time with P.hordei using 30mg uredospores per g talc. All plants were incubated in a saturated atmosphere for 24h and then transferred to a filtered-air cabinet. Four days after inoculation with P.hordei, all plants were inoculated with E.graminis and replaced in the filtered-air cabinet. After a further 10 days the number of mildew colonies and rust pustules per leaf were recorded. Mildew colonies were counted again after another 6 days.

The results (Table 14) confirmed that pre-inoculating leaves with large amounts of P.hordei considerably reduced mildew. They also showed that the percentage reduction from the appropriate controls of the mean number of mildew colonies per leaf was greater 16 days than 10 days from inoculation with E.graminis.

TABLE 14 (See also Appendix Table 17) Dual infections of rust and mildew: effects on mildew of pre-inoculating leaves with three different inoculum levels of P.hordei.

No. of inoculations with P.hordei	Mean number of mildew colonies per leaf. Nil (Control)	1	2	3
	(0)	(25.3)	(84.1)	(286.5)*
Assessment of mildew				
10 days	63.9	65.8	56.7	43.0
16 days	83.9	91.2	62.5	33.4
after inoculation.				
% reduction in mildew of control at:				
10 days	-	-3.1	11.2	32.7*
16 days	-	-8.7	25.5**	60.2***
after inoculation.				

* Figures in brackets indicate mean number of rust pustules formed per leaf.

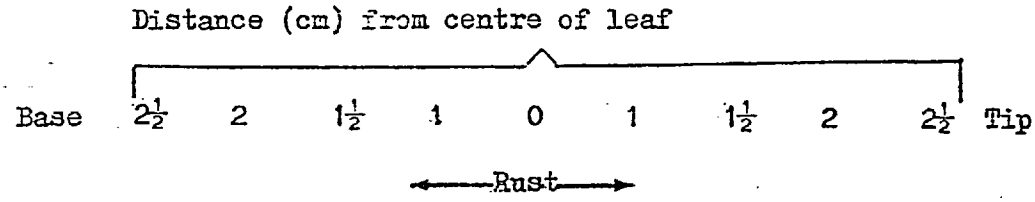
Significant differences from control of $P \leq 0.05$, 0.01 and 0.001 indicated by *, ** and *** respectively.

3. Effect on mildew of pre-inoculating a central band of the leaf with P.hordei.

Two pots each of 10-day-old Zephyr and Julia barley were used. The seedlings in one pot of each cultivar were inoculated with P.hordei by brushing a uredospore/talc mixture in a 2cm band across the centre of each leaf. The seedlings in the other two pots were similarly treated with talc as controls. All plants were incubated in a saturated atmosphere for 24h and then returned to the greenhouse. Three days later they were inoculated with E.graminis. After a further 7 days in the greenhouse the mildew which had developed was examined. For this, the leaves were removed, cleared in methanol for 24h and, after a light staining (10-20min.) in lactophenol-cotton blue the mildew colony size, and conidiophore density were recorded. Mildew colonies were chosen from the centre; the edges, and at ½cm distances from the bands of rust. Similar recordings were made from control leaves. (Table 15)

On both Zephyr and Julia the size of the mildew colonies was smallest in the centre of the rusted region of leaf. With increasing distance from the rust infections the mildew colonies grew larger, as did the number of conidiophores, reaching the dimensions of those on control leaves 2-2½cm from the centre of the bands of rust. These effects were more pronounced on the distal than on the basal side of the rust infections.

Table 15 (see also Appendix Table 18) Dual infections of rust and mildew: effects on mildew development of pre-inoculating leaves with a central band of P.hordei.



a. Zephyr

	2½	2	1½	1	0	1	1½	2	2½
Mean colony size (mm^2)†	1.88	1.38	1.34	0.55	0.28	0.95	1.18	1.54	1.23
Mean no. of conidiophores††	70.7	61.7	50.8	32.9	15.0	42.2	35.0	46.0	46.3

b. Julia

	2½	2	1½	1	0	1	1½	2	2½
Mean colony size (mm^2)†	1.47	1.21	0.98	0.54	0.23	0.34	0.90	1.07	1.32
Mean no. of conidiophores††	45.9	49.4	43.0	16.3	9.3	10.0	23.5	29.5	46.1

† Values for leaves not pre-inoculated with P.hordei: Zephyr 2.20mm^2; Julia 1.37mm^2.

†† No. per microscopic field at centre of colonies in central region of leaves not pre-inoculated with P.hordei: Zephyr 71.7; Julia 45.7

4. Effects on the Development of Mildew.

This experiment was designed to determine the stage in development at which mildew was affected by pre-inoculating leaves with P.hordei.

Five pots of 10-day old Zephyr barley seedlings were inoculated with P.hordei (group A) then, together with five pots of uninoculated seedlings (group B) were incubated in a saturated atmosphere for 24h. All seedlings were inoculated one day later with E.graminis so that there was a period of two days between the inoculations. The plants were then returned to a filtered-air cabinet for the remainder of the experiment. After 1, 3 and 6 days from inoculation with E.graminis five leaves were sampled at random from each of the two groups (A &B) of plants, impressions were made of those sampled at 1 and 3 days while those taken after 6 days were cleared in methanol and lightly stained in lactophenol-cotton blue. In addition, on the 8th day from inoculation with E.graminis ten leaves were taken from both groups of plants (A & B) and each group of ten leaves was used to provide inoculum in the apparatus shown in Fig. 1 to separately inoculate two further sets of healthy seedlings. The number of mildew colonies that developed on these secondary inoculated plants was recorded after a further 7 days.

From the stained leaf impressions the percentage of the conidia that germinated and formed appressoria was determined (Table 16a). Also from the leaf impressions made 3 days from inoculation with E.graminis was determined the percentage of the conidia that established

Table 16 (see also Appendix Table 19) Dual infections of rust and mildew: effects on mildew development of pre-inoculating leaves with P.hordei.

Mildew development on upper leaf surface.

		Mean % germination of conidia	Mean % Appressoria formation	Mean % establishment
a. Leaf impressions taken 1 day	A	20.24	72.00	-
	B	15.18	64.00	-
and 3 days	A	16.23	69.7	54.00
	B	18.07	81.70	53.90

after inoculation with E.graminis.

		Mean area of mildew colonies (mm^2)	Mean no. of conidiophores per central field.
b. Leaves sampled 6 days after inoculation with E.graminis.	A	1.59**	25.10***
	B	2.33	52.52

		Mean number of mildew colonies per cm^2 on 'secondary' plants.
c. Leaves sampled 8 days after inoculation with E.graminis, used to provide fresh inoculum.	A	3.302*
	B	5.651

A = Leaves pre-inoculated with P.hordei; B = leaves not pre-inoculated.

Values significantly different from B (control) at $P \leq 0.05$; 0.01; and 0.001 denoted by *; ** and *** respectively.

colonies (Table 16b). The area of these colonies and the number of conidiophores per field were determined from the cleared and stained leaves sampled after 6 days (Table 16c).

Percentage germination, appressoria formation and establishment of E.graminis appeared to be the same on control (B) and leaves pre-inoculated with P.hordei (A). The size of mildew colonies and the mean number of conidiophores per field on pre-inoculated leaves were significantly less than on the controls. This reduced production of conidia was reflected by the result that significantly fewer mildew colonies were produced by inoculating fresh plants with leaves from group A pre-inoculated with P.hordei than with leaves of group B not pre-inoculated with P.hordei. This effect was similarly shown by scanning electron microscopy of fresh material.

5. Effects on Rust and Mildew Development of Successive Periods of Competition.

The effects on the development and productivity of P.hordei and E.graminis of successive periods of competition are the subjects of the following experiment.

Two fungicides: ethirimol (ICI Plant Protection Ltd.) and Calirus (BAS 3170F BASFLtd.) were used to enable the examination of the development of rust and mildew (from the same inoculations) both in isolation and in combination.

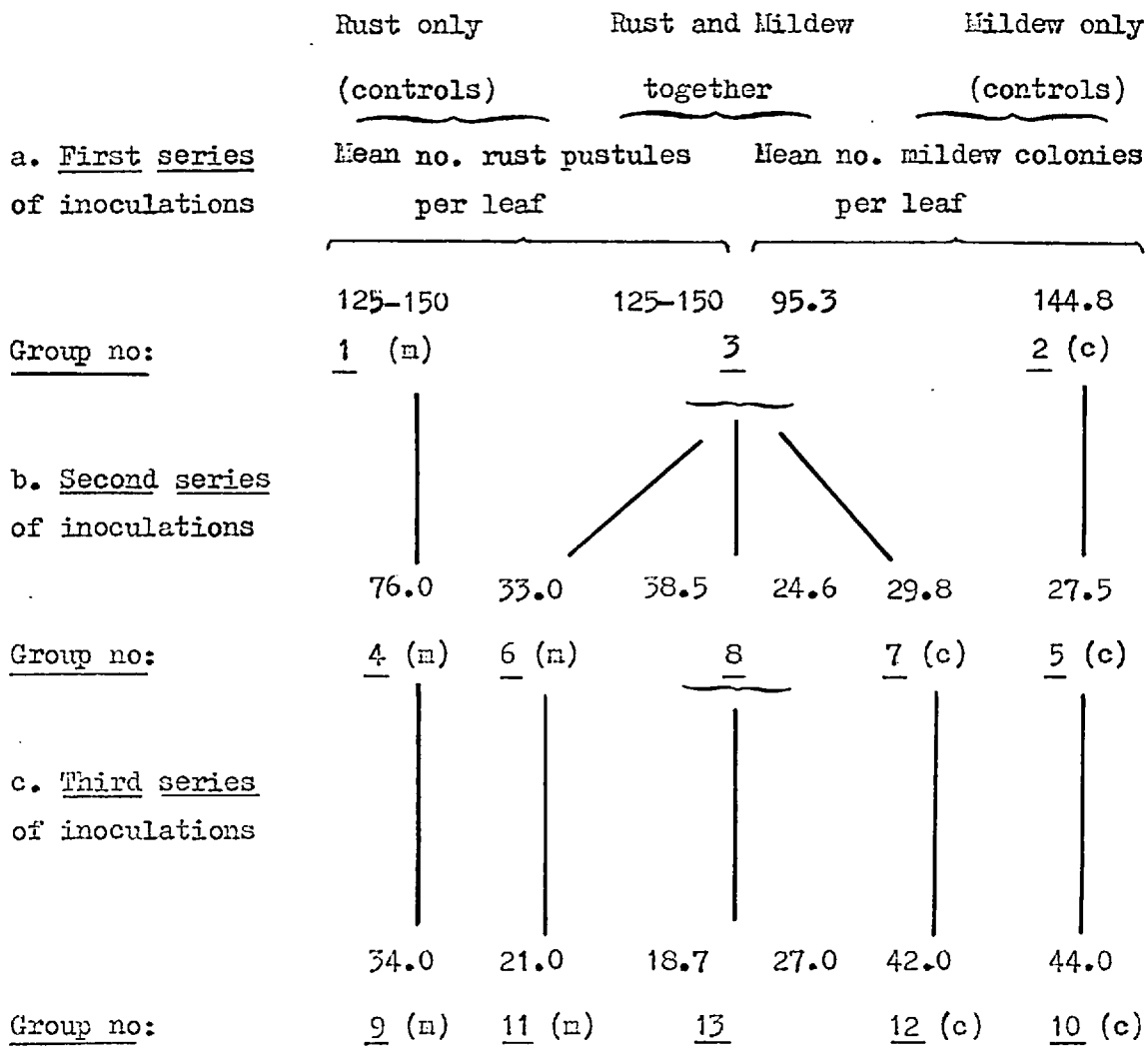
There were three series of inoculations, in the first six pots of 10-day old Zephyr barley (two of which were sown with milstem-dressed seed, group 1) were inoculated with P.hordei and immediately afterwards with E.graminis. The plants were incubated for 24h in a satur-

ated atmosphere, and two pots of seedlings (group 2) were sprayed with Calirus (0.01% in 0.0025% cittowet). This gave three groups of plants; group 1 (milstem-dressed seed) developed rust only; group 2 (sprayed with Calirus) developed mildew only, and group 3 (no chemical treatment) developed both rust and mildew. The amount of mildew and rust on these plants was assessed after 7 and 10 days respectively (Table 17A)

After seventeen days from the initial inoculations ten leaves were taken from each of the three groups of plants (shaken 48h previously) and the leaves from each group were then used (to provide inoculum in the inoculation chamber Fig. 1) to separately inoculate further groups of 10-day old Zephyr seedlings. Group 1 inoculum was used to inoculate two pots of milstem-dressed seedlings (group 4); group 2 inoculum to inoculate two pots of seedlings which were sprayed with Calirus 24h after inoculation (group 5), and group 3 inoculum to inoculate six pots of seedlings which were divided into three groups of two pots which received treatments corresponding to groups 1, 2 and 3, (two were sown with milstem-dressed seed (group 6), two were sprayed with Calirus 24h after inoculation (group 7), and two had no chemical treatment (group8)). Again all plants received the standard incubation in a saturated atmosphere and Calirus was sprayed onto the appropriate plants, (groups 5 and 7). The plants were then housed in filtered-air chambers so that groups of plants did not transfer inoculum, and the rust and mildew was assessed as before from the plants in groups 4 to 8. (Table 17B)

After a further seventeen days from the second series of inoculations a similar procedure was performed using inoculum from plants in

Table 17 Dual infections of rust and mildew: effects on rust and mildew of successive periods of competition.



(m) = milstem dressed seed sown

(c) = plants sprayed with Calirus.

Significant differences were obtained in the numbers of rust pustules per leaf between groups 9 and 11; 9 and 13; and 4 and 6 all at $P \leq 0.05$. Also in the number of mildew colonies per leaf in groups 12 and 13, 10 and 13 at $P \leq 0.001$ and 2 and 3 at $P \leq 0.05$.

groups 4 to 8 to inoculate fresh seedlings in groups 9 to 13. The results of the disease assessments made after a further 10 days are shown in Table 17c.

The results showed that where P.hordei and E.graminis were inoculated onto the same leaf and allowed to develop for 17 days both had a reduced capacity for propagule production when compared with the appropriate controls.

Rust in the experiment appeared to have been the less successful of the two pathogens in competition, but with different cultivars; races of P.hordei or E.graminis; periods of competition, or initial disease intensity the balance could have been altered.

With 17 days between the generations many of the leaves were beginning to senesce, and where higher disease levels were recorded the senescence was obviously more pronounced. This differing degree of senescence from one group of plants to another accounted for several of the anomolies seen in the results. Leaves with low levels of disease when used to provide inoculum resulted in proportionately higher disease in the following generation than leaves with heavy infections but correspondingly greater senescence.

The amounts of mildew on plants in groups 2, 5, 7, 10 and 12 were considered to be too low. This was because they were all sprayed with Calirus after inoculation and the growth of the mildew in such situations appeared to be inhibited.

1 (d) Dual infections of rust and mildew: causes of observed effects.

It was shown in the previous experiments that dual inoculation of leaves with E.graminis and P.hordei altered the course of development of both pathogens. The causes of these alterations were investigated in the following series of experiments:

(1) Effect on mildew of a subsequent inoculation of leaves with P.hordei.

One effect observed in previous experiments was that development of E.graminis was reduced on leaves which were subsequently inoculated with P.hordei. The following experiments attempted to determine whether this effect on mildew resulted solely from the subsequent development of rust or could be attributed to the process of inoculating the leaves with P.hordei, which involves the use of a water spray and the incubation of plants in a saturated atmosphere for 24h. The latter seemed a distinct possibility in view of the well-known inhibitory effect of free water on conidial germination of powdery mildew

Fifteen pots of Zephyr seedlings were inocubated with E.graminis and were then immediately divided into five groups of three pots. The seedlings in one group received no further treatment (treatment 1 of Table 18, control), those in another group received the standard procedure, for inoculating plants with P.hordei, i.e. a water spray, dusting with a uredospore-talc mixture and incubation for 24h in a saturated atmosphere (treatment 5, Table 18). The remaining three groups received respectively, a water spray followed

TABLE 18 Dual infections of rust and mildew: effects on mildew of a subsequent inoculation of leaves with P.hordei.

Treatment No.	Treatment.				
	Water	Talc	Uredospores in talc.	24 h saturated atmosphere	Mean mildew colonies/leaf.
1	-	-	-	-	58.7
2	+	+	-	-	54.8
3	+	+	-	+	45.1
4	-	-	-	+	40.9
5	+	-	+	+	34.1

by dusting with talc only, incubation in a saturated atmosphere only and both these treatments (treatments 2, 4 and 3, Table 18). After the appropriate treatment each group of pots was returned to a filtered air cabinet for 7 days when the number of mildew colonies on each leaf was counted.

Significant differences were obtained between treatments: 1 and 3; 1 and 4; and 1 and5. No significant differences were obtained between treatments: 3 and 4; 4 and 5; and 1 and 2. These results indicate that although a water spray in the inoculation process had little effect on mildew development, a period of 24h incubation in a saturated atmosphere significantly reduced mildew levels. Inoculation with uredospores appeared to have caused a further reduction in mildew but not significantly more than the humidity treatment.(cf. treatments 3 and 5)

2. Effects on P.hordei of pre-inoculating leaves with E.graminis.

E.graminis grows mainly on the leaf surface, its internal colonisation of the leaf being restricted to the haustoria which develop within some epidermal cells. In contrast, after the uredospores of P.hordei germinate and the germ tubes enter stomata, growth of the fungus occurs mainly within the leaf until eventually the epidermis is ruptured and pustules appear. The sites at which these fungi develop are thus different but this does not exclude the possibility that the effects of dual inoculations result from the physical exclusion of one pathogen from its infection site. However, it seems likely that physical interference of the infection and development of E.graminis by pre-inoculating leaves with P.hordei would only occur if very many uredospores were used or if the period between inoculations exceeded 8 days, i.e. the minimum time for rust pustules to erupt. Since neither of these conditions obtained in the experiments described so far limitation of leaf area available for colonisation by E.graminis was considered unlikely to be the reason for the effects observed on mildew development of pre-inoculating leaves with P.hordei. On the other hand, in situations where leaves were first inoculated with E. graminis the rapid development of mildew on the leaf surface might well limit the sites subsequently available for P.hordei. Consequently the following experiment was carried out to determine the amount of space the mildew colonies occupied, and whether the leaf area covered, accounted for the observed reductions in rust. Measurements were also made of the size of the stomatal openings on mildewed and healthy leaves.

Ten pots of uniform 10-day old Zephyr barley seedlings were inoculated with E.graminis (race B72/27). After allowing the conidia to settle, the pots were placed in a filtered air cabinet together with ten pots of similar but uninoculated seedlings as a control.

After 4 days the seedlings in two pots from each group were inoculated with P.hordei. They were incubated for 24 h in a saturated atmosphere and then returned to a filtered-air cabinet. The numbers of rust pustules that developed on these plants were recorded 9 days later (Table 19). The seedlings in the remaining pots were used to provide the following leaf samples on days 4, 6, 8 and 12 after inoculation with E.graminis. Five leaves were taken at random from the inoculated seedlings, their areas measured and the number of mildew colonies per leaf recorded. These leaves were cleared in methanol, lightly stained in lactophenol-cotton-blue and examined microscopically to determine the size of the individual mildew colonies.

Five other leaves were taken from the seedlings with E.graminis and five from the control plants. Impressions of these leaves were immediately made using silicone rubber (Sampson, 1961) and positive impressions derived from these rubber negatives using clear nail varnish and sellotape. These were examined microscopically and the width of the stomatal apertures measured (Table 19c).

The results (Table 19a) showed that on leaves pre-inoculated with E.graminis, there was a 68.7% reduction in the number of rust pustules. At the time of inoculating leaves with P.hordei less than 1% of the leaf area was occupied by mildew colonies and 2 days later, when penetration by P.hordei would have been completed, still only

TABLE 19 Dual infections of rust and mildew: effects on *P.hordei* of pre-inoculating leaves with *E.graminis*.

(See also Appendix Table 22)

(a) Reduction in Rust.

	Mean no.rust pustules/leaf.
Leaves pre-inoculated with *E.graminis*.	13.4*
Leaves not pre-inoculated (control).	42.9

*Significantly different from control at $P \leq 0.001$ ('t'-test)

(b) Mildew development (on leaves not inoculated with *P.hordei*).

	Days after inoculation with *E.graminis*			
	4	6	8	12
Mean colony size (mm^2)	0.07	0.51	2.09	3.98
Total area of colonies (mm^2)	5.56	45.89	179.80	340.58
% leaf area covered	0.82	6.76	26.50	50.20

Mean leaf area: 6.79cm^2. Mean no. colonies/leaf: 86.2.

(c) Width of stomatal apertures (μ)

	Days after inoculation with *E.graminis*.			
	4	6	8	12
Leaves not inoculated (control)	1.01	0.94	0.84	2.53
Leaves inoculated with *E.graminis*	0.99	1.03	1.49	1.26*

* Significantly different from the control at $P \leq 0.01$ ('t'test).

6.76% of the leaf was covered with mildew. It seems extremely unlikely, therefore, that the mildew at this stage physically excluded P.hordei. Even 12 days after inoculation with E.graminis only 50% of the leaf area was occupied by mildew, a figure somewhat less than the percentage reduction in rust.

Stomata with apertures 1 μ wide or less were considered closed, and only after 12 days did any difference in the width of the stomatal apertures on mildewed and control leaves become apparent. Those on control leaves opened while those on mildewed leaves remained closed. However the effects of infection with E.graminis on stomatal apertures could not be properly evaluated. For this, recordings would have to be made throughout the day and night under all conditions of humidity, lighting and temperature. Also since it is uncertain whether germ tubes of P.hordei can penetrate closed stomata or not this line of investigation was not pursued further.

(3) Effect on rust of pre-inoculating leaves with E.graminis 6 days earlier and of treating plants with ethirimol.

The results of the previous experiment suggested that allowing E.graminis to develop on leaves for 4 days before inoculating them with P.hordei could have had little direct, physical effect on uredospore germination and subsequent penetration. This was examined further by allowing E.graminis to develop on plants for 6 days before inoculating them with P.hordei and at this stage also treating some plants with ethirimol thus stopping mildew development and its demands on the host nutrients, whilst still maintaining a physical barrier to the rust spores.

Six pots of 10-day old Zephyr seedlings were used, the seedlings in four pots (groups B & C) were heavily inoculated with E.graminis, and all six pots (groups A,B and C) returned to greenhouse conditions for 6 days. Then mildewed seedlings in two pots (group C) were given a root drench with ethirimol (100 ml./pot 0.01% a.i. as the hydrochloride) and 6 h later the seedlings in all six pots were inoculated with P.hordei. The seedlings were incubated in a saturated atmosphere for 24 h and the amount of rust assessed after a further 9 days, first by counting pustules visible to the naked eye and then by detaching the leaves, clearing them in methanol and counting pustules under the microscope. Five leaves were also selected from each group, a 3 cm section taken from the centre of each and a sample of rust pustules were measured. The results are shown in Table 20 and in further detail in Appendix Table 23.

TABLE 20 (See also Appendix Table 23) Dual infections of rust and mildew: effect on rust of pre-inoculating leaves with E.graminis 6 days earlier and of treating plants with ethirimol.

Treatment	Mean no. rust pustules/leaf by eye	Mean no. rust pustules/leaf microscopically	Mean size rust pustules(mm^2)
A Leaves not pre-inoculated.(control)	119.5[a***]	171	0.090[a**]
B Leaves pre-inoculated 6 days earlier with E.graminis	43	99[b*]	0.054
C Leaves pre-inoculated with E.graminis and also treated with ethirimol after 6 days.	98[a*]	123[b**]	0.104[a**]

a Significantly different from treatment B and b, significantly different from treatment A at $P \leq 0.05$ (*), 0.01 (**) and 0.001 (***) respectively, based on 't'-tests.

The results indicate that the reduction in the number and size of rust pustules due to pre-inoculation of leaves with E.graminis could be partly reversed by killing the mildew after 6 days with ethirimol. This suggests that both a physical barrier and a nutrient effect were involved in reducing the level of rust on leaves pre-inoculated with E.graminis. The slight increase in the size of the rust pustules (Treatment C) over the controls (Treatment A) could have been due to there being fewer rust pustules and no mildew colonies left alive to compete for nutrients.

(4) Effect on mildew development of pre-inoculating leaves with P.hordei and of treating plants with Calirus.

The implication that some nutritional competition was involved in the interaction between E.graminis and P.hordei suggested a further experiment of similar design. In this plants were first inoculated with P.hordei and rust allowed to develop for 8 days (Flecking Stage). These plants were then inoculated with E.graminis but some were also treated with Calirus (0.01% BAS3170F in 0.0025% citowett) to arrest further rust development. It was considered that effects in this instance might be more readily attributed to nutritional factors since rust infections are unlikely to constitute a physical barrier to the development of E.graminis on the leaf surface.

There were in all nine pots of 10-day old Zephyr seedlings. The seedlings in six pots were uniformly inoculated with P.hordei (groups B and C), the others in the remaining three pots were not (group A). After 8 days, three pots of inoculated seedlings

(group C) and those in group A were sprayed with Calirus. When the spray deposit had dried, the seedlings in all nine pots were inoculated with conidia of E.graminis. After a further 7 days the number of mildew colonies per leaf was counted, firstly by eye and then microscopically from five randomly selected leaves from each treatment. For this purpose the leaves were cut in half, cleared in methanol and lightly stained in lactophenol cotton-blue. Separate measurements were also made of the size of the mildew colonies and their conidiophore density for each half of each leaf.

The results (Table 21) indicate that where P.hordei was eradicated after 8 days (though only partly so in this experiment) the size of the mildew colonies and the density of their conidiophores were greater than on leaves where rust was allowed to develop fully. However the colonies did not attain the dimensions of those on the control leaves and these two statements together imply that competition for nutrients is involved in the interaction but that the extent of its involvement remains uncertain.

As observed earlier pre-inoculation of leaves with P.hordei had a greater effect on E.graminis in the distal regions of the leaves. In this experiment the effect was not on the number but on the size and sporulation of the colonies, though this resulted in fewer colonies being recorded when counts were made by eye.

Table 21 (see also appendix table 24) Dual infections of rust and mildew: effects on mildew development of pre-inoculating leaves with P.hordei and of treating plants with Calirus.

Treatment:		Mean no. mildew colonies per leaf by: eye	Mean no. mildew colonies per leaf by: microscope	Mean colony size (mm^2)	Mean no. conidiophores/field.
A. Leaves not pre-inoculated; sprayed after 8 days with Calirus.	Basal	20.6[a*]	26.0	2.50	34.1[a*]
	Distal	17.6[a*]	34.0	2.24[a**]	40.8[a**]
B. Leaves pre-inoculated with P.hordei; no Calirus spray	Basal	13.6	32.0	2.03	19.9
	Distal	3.2	28.6	2.30	7.0
C. Leaves pre-inoculated with P.hordei; sprayed 8 days after with Calirus.	Basal	22.4[a*]	28.8	2.50	31.5
	Distal	14.0[a*]	32.0	1.44	18.9[b*]

Mean number of rust pustules in treatment B, 113.6; in treatment C, 15.5.

a = significantly different from corresponding value in treatment B.

b = significantly different from control value at $P \leq 0.05(*)$ and $0.01(**)$.

5. Effects of materials likely to alter the carbohydrate content of leaves.

If the observed interactions between E.graminis and P.hordei result in part from a competition for nutrients by the two pathogens, as some of the experiments indicated, then treatments which affect carbohydrate levels of leaves might also be expected to affect these interactions. Two such treatments, floating leaf segments on sucrose solutions and treating plants with maleic hydrazide were investigated in the following experiments.

a) Floating leaf segments on sucrose.

There were two experiments. In the first, three pots of 10-day-old Zephyr barley were inoculated with P.hordei; three pots of similar seedlings were left uninoculated. After 5 days ten segments, 2.5 cm long, were cut from leaves inoculated with P.hordei, five of these were floated on distilled water (A) and five on 2% sucrose (B) contained in small, polystyrene boxes (55 x 35 x 20mm). Five leaf segments were also cut from the control plants; these were floated on distilled water (C). All leaf segments were then inoculated with E.graminis.

After a further 8 days in greenhouse conditions the leaf segments were cleared in methanol, stained in lactophenol-cotton-blue and the mildew colonies on them examined under the microscope. Those on leaf segments pre-inoculated with P.hordei were spreading with no easily defined boundaries which made area determinations unreliable. As an alternative conidiophores were counted in a microscopic field taken from the centre of five colonies on each

leaf segment.

The results, (table 22a) indicated that the reduction in the conidiophore density normally seen on leaves pre-inoculated with P.hordei could be reversed by an exogenous supply of sucrose.

In the second experiment, five pots of 10-day-old Zephyr barley were inoculated with E.graminis; five pots of similar seedlings were left uninoculated. Both sets of plants were kept for 6 days in a filtered-air cabinet and then they were inoculated with P.hordei, since in previous experiments direct inoculations of detached leaves were not successful. After a further 4 days, twenty-four leaf segments were cut from each set of plants; twelve of these were floated on water and twelve on 2% sucrose solution to give the treatments shown in Table 22b.

After a further 7 days in greenhouse conditions the leaf segments were cleared in groups of four in 10ml 96% methanol for 48 h in the dark. The areas of the rust pustules on the cleared segments were determined microscopically and the chlorophyll extracted by the methanol determined using a Beckman D.B. spectrophatometer (Mackinney, 1941).

The results (Table 22b) showed that rust pustules on segments pre-inoculated with E.graminis were much smaller than those on leaf segments not so pre-inoculated. This reduction in size of pustules was partly reversed by supplying sucrose to some leaf segments but this treatment accelerated the loss of chlorophyll. In contrast, mildew delayed the loss of chlorophyll from the leaf segments.

TABLE 22 (Seealso Appendix Table 25) Dual infections of rust and mildew: effects of sucrose.

(a) Leaf segments inoculated with *E.graminis*

	Pre-inoculated with *P.hordei*.		Not pre-inoculated.
	floated on water (A)	floated on 2% sucrose (B)	floated on water (C)
Mean no. conidiophores/field. (25fields x 0.045mm^2)	8.1[a]	17.2[a]	21.8[a]

(b) Leaves inoculated with *P.hordei*

	Pre-inoculated with *E.graminis*, then leaf segments floated on		Not pre-inoculated then leaf segments floated on	
	water	2% sucrose	water	2% sucrose
Mean area of rust pustules (mm^2)	0.013[b]	0.063[b]	0.206[b]	0.220
Total chlorophyll (mg/l)	5.547	5.020	2.250[c]	1.467[c]

a, differences between means significant at $P \leq 0.01$

b, differences between means significant at $P \leq 0.001$

c, significantly different from water control at $P \leq 0.001$.

(b) Treating plants with maleic hydrazide.

There were again two experiments. In the first experiment there were six pots of 10-day-old Zephyr barley. The seedlings in four pots were inoculated with P.hordei (A and B), those in the other two pots were not (c). All plants were kept in a saturated atmosphere for 24 h and then two pots of inoculated seedlings (B) were treated with maleic hydrazide by applying 50ml of a 0.1% solution to the soil surface in each pot. This treatment was repeated on the three following days and then the seedlings in all six pots were inoculated with E.graminis. After a further 7 days in the greenhouse, the number of mildew colonies that had developed on each leaf was recorded.

The results (Table 23a) showed that ~~the~~ like supplying sucrose to leaf segments the reduction in mildew due to pre-inoculating leaves with P.hordei could be reversed by treating the plants with maleic hydrazide.

The second experiment was essentially similar in design but two cultivars, Zephyr and Julia, were used, the size of the resulting mildew colonies was measured 10 days after inoculation with E.graminis and the number of colonies per unit area was determined microscopically.

The results (Table 23b) confirmed those of the previous experiment. Fewer rust pustules on Julia (due to its resistance to P.hordei) were associated with larger mildew colonies.

TABLE 23 (See also Appendix Table 26) — Dual infections of rust and mildew: effects of maleic hydrazide.

(a) Leaves inoculated with E.graminis.

	Pre-inoculated with P.hordei.		Not pre-inoculated.
	No further treatment(A)	treated with maleic hydrazide(B)	(C)
Mean no. mildew colonies/leaf.	36	72^{***} - ns -	83^{***}

(b) Leaves inoculated with E.graminis.

	Pre-inoculated with P.hordei		Not pre-inoculated.
	No further treatment(A)	Treated with maleic hydrazide(B)	(C)
Mean no. mildew colonies/leaf on			
Zephyr	30	75^{***} - ns -	76^{***}
Julia	34	67^{***} - ns -	69^{***}
Mean area of colonies(mm^2) on			
Zephyr	1.02	2.85^{**} - $P \leq 0.001$ -	5.78^{***}
Julia	2.29	4.81^{**} - ns -	5.27^{***}

Significant differences from values for treatment A at $P \leq 0.01$ and 0.001 indicated by ** and *** respectively. Differences between treatments B and C indicated in body of table. (Based on 't' tests)

Infections of rust and mildew: alterations in host components.

The aim of these experiments was to determine whether the effects observed when leaves were inoculated in turn with the two pathogens could be related to any obvious changes in host components.

Because it is difficult to obtain the same disease levels in consecutive experiments it was decided first to perform a series of determinations on one set of experimental material. The procedure used is outlined below. It must be emphasized that the purpose of this experiment was to compare the levels of the various components with and without infections of rust and mildew and to find out when changes occurred rather than to determine absolute quantities.

Seventy-five pots of Zephyr barley were grown in John Innes No 2 potting compost. They were divided into five groups(A to E) each of fifteen pots with ten to twelve seedlings per pot, which were treated as follows: A, uninoculated control; B, inoculated with P.hordei and C, with E.graminis 10 days after sowing; D, inoculated with P.hordei on day 10 and with E.graminis on day 13 after sowing; E, inoculated with E.graminis on day 10 and with P.hordei on day 13 after sowing.

The following methods were then used:

1. Eleven days from sowing twenty-five leaves were sampled at random from each treatment. Similar samples were taken every 3 days.
2. The amounts of rust and mildew on these leaves were assessed (Appendix Table 27)
3. Each sample was divided into four sub-samples of five leaves which were weighed. One sub-sample of leaves was dried at 70°C for 3 days and reweighed. (Fig 2 and Appendix Table 28)
4. The remaining sub-samples of leaves were each macerated for 3 min. in 15ml 80% ethanol in a Sorval Omnimixer cooled in ice.

5. The macerated tissue was centrifuged for 10 min at 3500 rpm(950g) and the supernatant decanted off. The precipitate was resuspended in 80% ethanol, recentrifuged and the supernatants from these operations were combined for each sub-sample and made up to 40ml with 80% ethanol. The optical density was then recorded using a Beckman D B spectrophotometer at 665nm and 650nm to determine chlorophylls a and b.(Fig 3 and Appendix Table 29)

6. The recombined supernatant was extracted with an equal amount of diethyl ether. The aqueous phase was separated and used for the determinations of water soluble carbohydrates by the Anthrone method. (Fig 4 and Appendix Table 30)

7. The precipitate from (5) was suspended in 20ml phosphate buffer at pH 7.4 and divided into two 10ml aliquots. The first was stored and used later to determine protein content by the Lowry method (Fig 5 & Appendix table 31)

8. Five ml of the second aliquot were taken and added to 5ml of 5% sodium lauryl sulphate in Tris-buffer and sodium citrate. The mixture was shaken and heated on a water bath for 15min at 60°C. After cooling 10ml of a chloroform/isc-amyl alcohol mixture 24:1 were added, the mixture was shaken for 15min on a mechanical shaker and centrifuged for 10min(950g)

9. The mixture so treated separated into three distinct layers. Five ml were taken from the top layer using a pasteur pipette, and diluted to 20ml with warm (60°C) distilled water. The nucleic acid in this fraction was determined from the absorbance at 260 nm on a Beckman D B spectrophotometer (Appendix Table 32) (Methods in Enzymology vol. III)

10. Calibration curves were prepared using: sucrose(analar BDH Ltd.) reacted with Anthrone reagent, for water soluble carbohydrate determinations; albumen(BDH Ltd.) in phosphate buffer, for protein determinations; and RNA(BDH Ltd.) in 0.625% sodium lauryl sulphate in Tris-buffer and

sodium citrate, for nucleic acid determinations.

The development of P.hordei and E.graminis from single inoculations of leaves followed the usual patterns. Mildew developed more slowly on leaves also inoculated with (before or after) P.hordei.

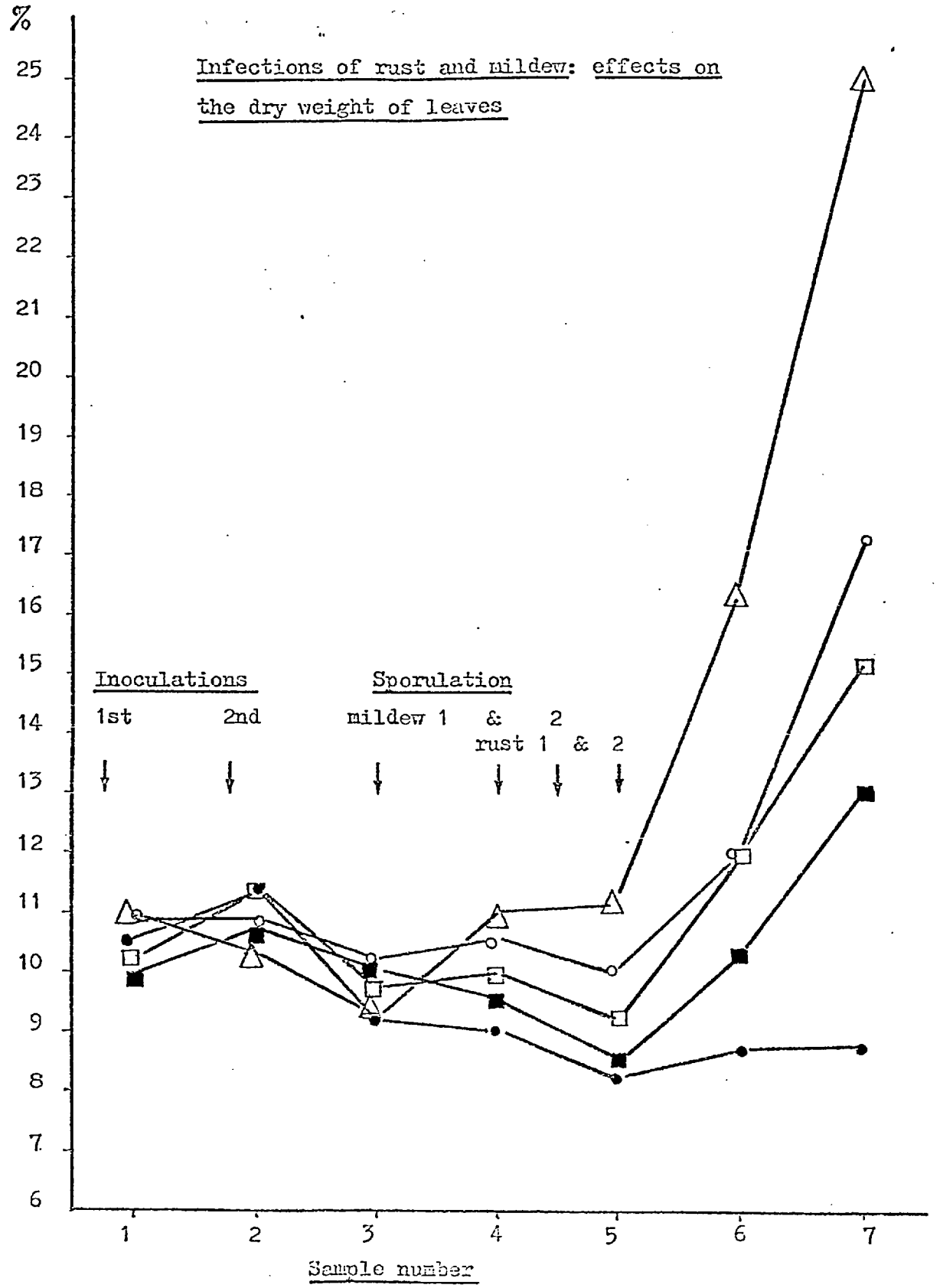
Both rust and mildew had a drying effect on the leaves. Although this effect did not become pronounced until 17 to 20 days after inoculation it is probably an indication of a greater water stress in diseased leaves earlier than this. Rust and mildew also both reduced the chlorophyll content of leaves, mildew particularly so. The reduction in chlorophyll was generally greater in leaves infected with both pathogens and was most rapid following sporulation.

There were also marked changes in water soluble carbohydrate, protein and nucleic acid content of leaves. Carbohydrates increasing considerably in mildewed leaves, reaching a peak 9 to 10 days after inoculation in all treatments (C, E and D) where mildew developed. The carbohydrate level then declined in treatment C (E.graminis only) to the level of that in the control and below this in treatments D and E (E.graminis and P.hordei). The increases in carbohydrate with rust were much smaller and reached a peak 12 to 13 days after inoculation. Peaks in carbohydrate levels with each pathogen corresponded with sporulation and the subsequent decline was probably related to the loss of spores and accelerated senescence.

The total protein content of leaves was also increased in infected leaves. These increases probably reflect in part the growth of the pathogens and showed the largest increases over the control 7 to 10 days after inoculation. The results of nucleic acid determinations were variable, and although increases were detected with infections of E.graminis and P.hordei no definite conclusions could be made. since the extraction was not sufficiently precise for accurate determinations.

Dry weight

Figure 2.



Key: ● = control uninoculated leaves.

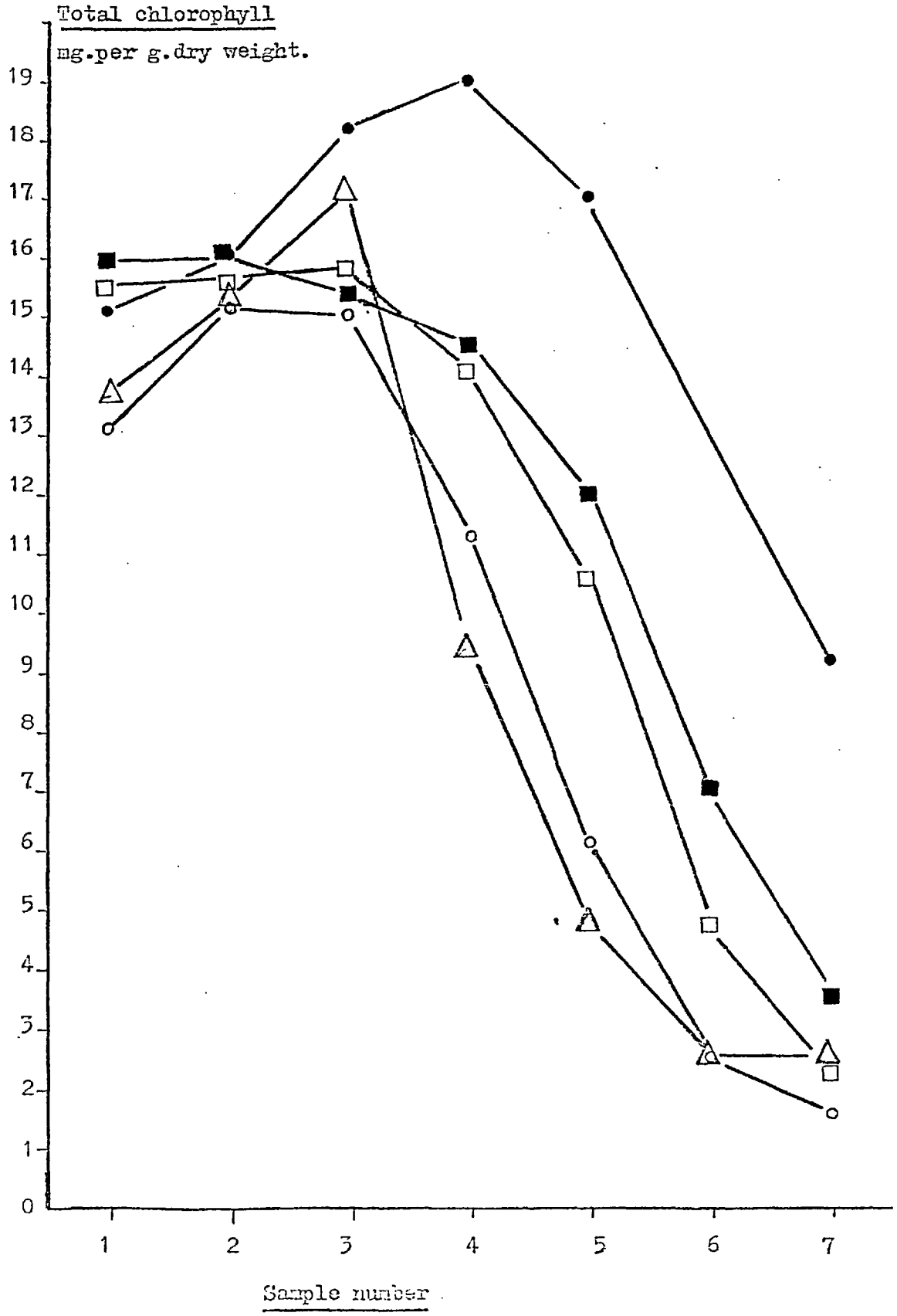
■ = leaves inoculated with P.hordei only

○ = leaves inoculated with E.graminis only

□ = leaves first inoculated with P.hordei and then E.graminis

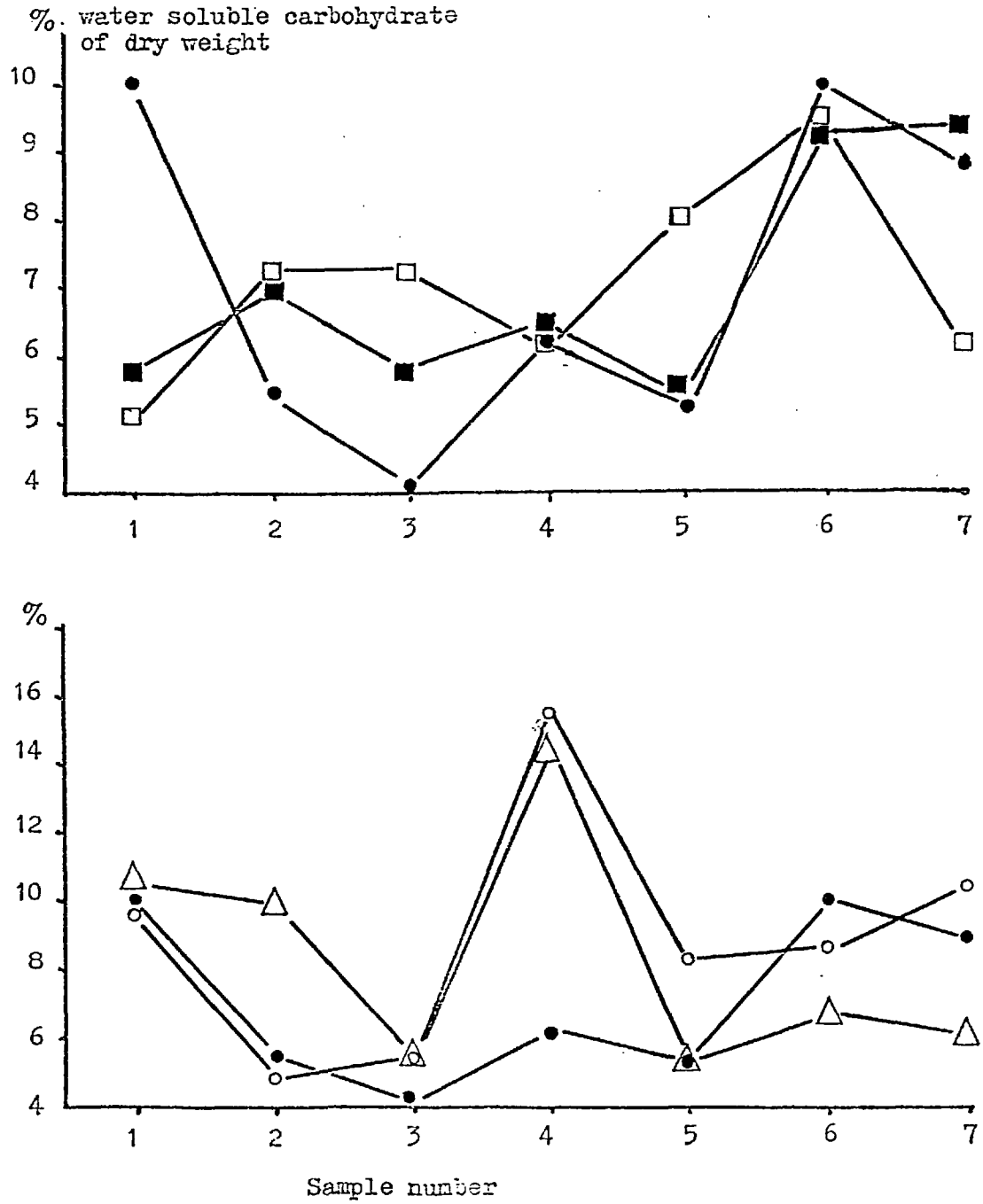
△ = leaves first inoculated with E.graminis and then P.hordei

Figure 3 Infections of rust and mildew: effects on the chlorophyll content of leaves.



Key : as in Fig 2

Figure 4 Infections of rust and mildew: effects on water soluble carbohydrate content of leaves.



Key : as in Figure 2

The dying of leaves (senescence), both naturally and as a result of infection makes it difficult to interpret the results. Clearly both P.hordei and E.graminis severly disrupt the host metabolism; causing increased production of carbohydrate and protein at a time when the chlorophyll levels were declining and the water stress was high.

Although most experiments were performed using periods between the inoculations of six days or less(when the result show there to be little disruption in the host due to the'first' inoculation), the period 7 to 10 days afterwards, when the 'second' disease was begining to develop would have been the period of maximal upheaval within the host.

The marked changes observed in the water soluble carbohydrate of leaves infected with E.graminis prompted the following experiment, which aimed to compare the carbohydrate within the colonies (including the surrounding leaf tissue to a radius of 1-2 mm) to that in uninfected regions of the same leaves.

Fifteen pots of 10-day old Zephyr barley seedlings were used, the seedlings in five were inoculated with P.hordei, those in another five were lightly inoculated with E.graminis, those in the remaining five plots were left uninoculated as controls. After 12 days under greenhouse conditions leaves from each of the three groups of plants were cut off. Three replicate portions(1g.each) from each of the three groups of plants were immediately weighed and three similar samples dried for 3 days at 70°C and then weighed. The remaining rusted and mildewed leaves were taken, the lesions isolated using a sharp scalpel, and collected(this included leaf tissue under the lesions and around them to a distance of 1-2 mm). The regions of the leaves that remained were also collected but areas greater than $1cm^2$ where little disease occurred, and senescent tissue were discarded. The fresh and dry weights

of each type of tissue were again determined from three replicate samples. Each replicate sample, from whole and dissected leaves,(see Table 24) was deep frozen (at -14° to -20° C) and then individaully macerated using a pestle and mortar. The macerates were extracted with 50% ethanol and filtered using a buchner funnel. The filtrates were diluted with 50% ethanol to 10ml and 0.2 ml of each used to determin the quantity of water soluble carbohydrate by the Anthrone method. The remaining extracts of the whole leaves (rusted, mildewed and control leaves) were desalted using ion exchange resin (Biodemineralite 20% w/v) for 2h. They were then filtered and rotary evaporated under vacuum (at 40°C) to 1ml. 0.1ml of each was then applied to thin layer plates of Silica Gel and chromatographed using N-butanol: water: acetone(4:5:1) as the solvent system. A standard solution containing known sugars was similarly treated and the resulting dried plates were sprayed with Aniline-diphenylamine reagent (reagent 8, Stahl 1969) and developed by heating at 85°C for 10min.

The results (Table 24) showed that the carbohydrate content of infected leaves was higher than in the surrounding tissues. In turn the level in these regions was higher than in uninoculated control leaves. These increases were in quantity of existing carbohydrates; the types detected in diseased leaves were not different from those in healthy leaves (Appendix Table 34). Extracts from mildewed leaves produced darker and larger spots on thin layer plates for glucose, sucrose and maltose than for any other sugars. The increase in maltose is interesting since it probably arose from the hydrolysis of starch and could indicate the extent of the sugar utilization in mildewed leaves.

In all, thirteen sugars were detected; of those identified (maltose, galactose, sucrose, fructose, glucose and ribose) only the extracts from rusted leaves were different. In these extracts maltose was not

detected. The other sugars detected were found to be the same in extracts from diseased leaves and those from healthy leaves and thus did not warrant further identification here.

Infections of mildew on detached leaf segments floated on different concentrations of glucose.

The results of the previous experiment indicated that the level of carbohydrate was higher in the uninfected regions of diseased leaves than it was in the uninoculated control leaves. This implied that there was an increase, rather than the depletion of carbohydrate expected from the results of earlier experiments(page67). Thus the conclusion that the lack of carbohydrates was limiting the growth and development of the second pathogen, was obviously in some doubt. In fact a suggestion was put forward that it was the increase in carbohydrate in leaves pre-inoculated with P.hordei that was limiting to the growth of the mildew. This was interesting since it was known that rose powdery mildew (Sphaerotheca panosa) could be controlled by sugar applications(Russell, 1973), and this prompted the following experiment. The aim of this experiment was to determine the concentration of glucose required to inhibit the growth of E.graminis and to compare this with the level determined from the uninfected regions of rusted leaves in the previous experiment.

Twenty leaf segments (2.5 cm long) from the first leaves of Zephyr seedlings were floated on each of five glucose solutions (2,4,6,8, and 10%) contained in small polystyrene boxes. A set of

Table 24 (see also Appendix Table 33) — Infections of rust and mildew: alterations in the water-soluble-carbohydrate content of leaves.

	Weight of tissue used for extraction (g.)	Mean % water soluble carbohydrate per g. dry weight.
Healthy leaves	1.0	2.102
Rusted leaves	1.0	2.330
Mildewed leaves	1.0	3.551
Isolated rust pustules and surrounding tissue	0.05	7.174
Remaining parts of rusted leaves	0.05	4.392
Isolated mildew colonies and surrounding tissue	0.2	3.317
Remaining parts of mildewed leaves	0.2	3.089

Table 25 (see also Appendix Table 35) — Infections of rust and mildew on detached leaf segments floated on a range of concentrations of glucose.

Glucose concentration (%)	Mean no. of mildew colonies per segment	Probability of difference.
0 (control)	102.5	-
2	83.7	n.s.
4	54.2	0.001
6	50.1	0.001
8	28.2	0.001
10	0.4	0.001

leaf segments were similarly placed on distilled water as a control (table 25).

The boxes were arranged randomly in the inoculation chamber and the leaf segments dusted with conidia of E.graminis. After 8 days the numbers of mildew colonies were recorded using a binocular microscope.

The results (Table 25) showed that concentration of glucose as low as 2% reduced the number of mildew colonies. however, reductions in the mildew that were comparable to those caused by pre-inoculation of leaves with P.hordei were not observed until the concentrations of glucose used were as high as 6 to 8%. The level of water soluble carbohydrate in the non-diseased regions of rusted leaves was shown to be 0.84% of the fresh weight in the previous experiment, and the highest level recorded for rusted leaves in an earlier experiment (page77) was less than 2% of the fresh weight. This indicated that the increased carbohydrate due to infection of leaves with P.hordei was unlikely to be the cause of the reduction in mildew.

Infection of leaves with E.graminis: effects on host components-antifungal compounds.

In the situation where both P.hordei and E.graminis are virulent obligate pathogens inoculated onto the same leaves, it was considered unlikely that phytoalexins were involved in bringing about the observed reductions in the disease levels. This does not exclude the possibility that pre-formed antifungal compounds influence the course of events when leaves are first inoculated by one pathogen and then by another. The presence of such compounds (Hordatines)

was shown by Stoessl & Unwin, (1970) when they isolated compounds (reported to have considerable antifungal activity) from healthy barley leaves.

Whether these compounds were effective against P.hordei or E.graminis, or whether they were altered in activity or amount by infections of E.graminis are the subjects of the present section.

Two extraction procedures were followed using the first leaves of Zephyr seedlings infected with E.graminis. The first method was that used by Stoessl and Unwin,(1970) and the second was as shown in the flow diagram (fig 5) based on work done on the extraction of antifungal compounds from wheat (Baker, 1975).

In both cases as a result of inoculating the first seedling leaves of Zephyr barley with E.graminis race B 72/27 10 days earlier the leaves had 70 to 80% cover of mildew.

In the first method 25g. of leaf tissue were boiled in 100ml distilled water for 10 min, filtered through muslin and then shaken with amberlite ion-exchange resin (IRC 50 H^+ 5g.resin to 100ml.) for 1h. The resin was recovered by filtration washed with 100ml.distilled water and then shaken for 1h. in 100ml. 2 N. acetic acid to elute the adsorbed bases. The resin was filtered and the filtrate concentrated to 5ml. under vacuum at 40°C. Portions of the concentrate were applied to thin layer plates of silica gel using n-butanol : water : acetic acid (4:5:1) as the solvent system.

In the second extraction procedure 50g of mildewed Zephyr leaves were extracted to give the four fractions denoted in the flow diagram. Each was concentrated to 5ml and drops placed on thin layer plates of silica gel. The solvent systems used were :- methanol : chloroform : water (45:45:10); n-butanol: water : acetic

Figure 5 Extraction Procedure 2

Flow diagram:

1 Macerate tissue in 96% (hot) Ethanol in Sorvall Omnimixer.

2 Make to 80% Ethanol and filter under vacuum.

3 Evaporate under vacuum at 40°C until almost dry. Adjust to 60% Ethanol and shake three times with equal volumes of Petroleum Ether(40-60°C Bpt.) Separate layers.

4 Evaporate aqueous/ethanol fraction to dryness and take up in distilled water. Shake three times with Diethyl Ether and separate.

Concentrate Petroleum Ether fraction and chromatogram. (1)

5 Evaporate aqueous fraction to less than 5ml and shake three times with N-butanol. Separate layers.

Evaporate Diethyl Ether fraction to almost dryness and chromatogram. (2)

6 Concentrate N-butanol fraction and chromatogram. (3)

Concentrate aqueous fraction and chromatogram. (4)

acid (4:5:1); and ethyl acetate : cyclohexane (1:1), for aqueous, n-butanol and ether fractions respectively.

The r.f. values of the U.V. absorbing compounds were recorded, and part of the first extract was used to determine the U.V. spectrum, this was found to be similar to that given by earlier workers for the 'Hordatine' fraction.

The presence of antifungal materials on the dried plates was determined by spraying the plates with a spore suspension of Cladosporium sp. (in 2% glucose and 1% Bacteriological peptone) and incubating them in a saturated atmosphere for 10 days.

The plates containing the products from the first extraction procedure (Stoessl & Unwin, 1970) exhibited antifungal activity in a region that corresponded to the r.f. values given for the hordatines, but no consistent antifungal activity was detected on the plates where the products of the second extraction were tested. Work on the second procedure as a consequence of these results was discontinued.

Further extracts of the hordatines were prepared using leaf tissue (25g.) heavily infected with E.graminis for 10 days and a similar quantity from healthy (uninoculated) leaves. The extracts were tested for inhibitors of germination using uredospores of P.hordei and conidia of E.graminis. Each extract was diluted with distilled water to give a range of concentrations; there was also a distilled water control. Three drops of each were placed on clean slides and covered with rectangles of porous cellophane(3 x 1½ cm), which had previously been boiled in distilled water for 30 min. Uredospores and conidia were then dispersed onto the cellophane using a settling tower. After incubating in a saturated atmosphere

for 24h, each piece of cellophane was transferred to a clean slide, stained with lactophenol-cotton-blue and the percentage germination of uredospores and conidia was assessed.

The results (Table 26) demonstrate that the extracted fraction (which corresponded to the hordatines of Stoessl & Unwin, 1970) from both the healthy and mildewed leaves caused similar inhibition of uredospore germination. The extract from the mildewed leaves appeared to show slightly greater activity in this respect.

The inhibition of conidial germination was far less pronounced and significant reductions from the control(distilled water) were only obtained at the highest concentration of both extracts.

Table 26 (see also Appendix Table 36) Infection of leaves with E.graminis: effects on hosts' components - antifungal compounds.

Spore germination tests on extracts from healthy and mildewed Zephyr barley.

Mean percentage germination.

	Control (distilled water)		Dilutions of extracts†			
			1	2	3	4
Uredospores of P.hordei	28.7	Healthy	1.16	4.98	13.92	37.95
		Mildewed	0.56	3.50	4.80*	22.70*
Conidia of E.graminis	42.0	Healthy	32.00	38.00	50.00	42.00
		Mildewed	24.00	39.00	34.00	35.00

* Values significantly different from the healthy dilutions at $P \leq 0.05$

† Dilutions:

1 = 2 x Tissue concentration (25g. tissue in 12.5ml)

2 = 1/5 Tissue concentration

3 = 1/50 Tissue concentration

4 = 1/500 Tissue concentration.

2. Interactions on cultivars with different levels of resistance to either P.hordei or E.graminis.

Introduction.

The results of the previous section indicated that when both P.hordei and E.graminis developed on Zephyr barley, a competition existed between the two fungi for the available nutrients within the leaf.

This section deals with interactions between P.hordei and E.graminis on different cultivars of varying degrees of resistance particularly Mazurka; a cultivar totally resistant to E.graminis race B 72/27.

Materials and Methods.

The barley cultivars used were; Midas and Julia, which were respectively more susceptible and more resistant to P.hordei than Zephyr; Mazurka, which was totally resistant to E.graminis race B 72/27, and Zephyr, which was used in experiments with these cultivars as a standard. In other experiments Zephyr was pre-inoculated with E.graminis race B 71/98 to which it was totally resistant; a situation analogous to the inoculation of Mazurka with E.graminis race B 72/27. In such experiments when cultivars were known to be resistant to a particular race of E.graminis, the initial inoculation density was assessed by allowing mildew to develop on susceptible cultivars, that had been included with resistant cultivars in the inoculation chamber.

The experimental conditions for growing the seedlings as well

as for their inoculation and incubation were the same as described in Section 1. So too were the techniques used for clearing, staining and examining the inoculated leaves.

Experimental.

Dual infections of rust and mildew on four barley cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis and P.hordei respectively.

Twenty-eight pots of seedlings of each of the four barley cultivars (Mida, Julia, Mazurka and Zephyr) were grown and 10 days after sowing, seedlings in seven pots of each cultivar (labelled group A) were inoculated uniformly with P.hordei. The seedlings in seven other pots of each cultivar (group B) were inoculated with E.graminis, while the remaining fourteen pots of seedlings (groups C and D) were not inoculated.

One pot of each cultivar from group A was taken at 0, 1, 2, 4 and 6 - days after inoculation with P.hordei and, together with one pot of uninoculated seedlings of each cultivar (group C) as a control was inoculated with E.graminis. A similar procedure was adopted with the seedlings of each cultivar in group B; one pot of these and the appropriate control seedlings from (group D) were inoculated at 0, 1, 2, 4 and 6 days (from inoculation with E.graminis) with P.hordei.

The resulting number of mildew colonies and rust pustules were recorded 9 days after the second inoculations with either E.graminis or P.hordei. The leaves were detached, and a central 5 cm section from each was cleared and stained and the size of the

mildew colonies and the rust pustules were measured.

Table 27 shows the mean number of mildew colonies and rust pustules that developed; Table 28 shows the mean colony/pustule, area, and Table 29, with figs. 6 and 7 represent the mean total area of rust pustules and mildew colonies per leaf expressed as percentages of the mean total area of pustules and colonies on the appropriate control leaves. In most situations, there appeared to be two components of disease development that were reduced as a result of dual inoculation of leaves with E.graminis and P.hordei, colony or pustule size and colony or pustule number. The exceptions were those where leaves were inoculated with E.graminis and then one day later or two days later with Midas they were also inoculated with P.hordei; in these situations, the number of pustules was often increased.

The patterns of the interactions differed in detail on the four cultivars, as might be expected since these differed in their resistance to both P.hordei and E.graminis, but there were certain features common to all cultivars. This is so in respect of the development of P.hordei on leaves first inoculated with E.graminis and is best shown by expressing total pustule area per leaf as a percentage of that on the control (Fig.6). With periods between the inoculations of 0 or 1 day total pustule area was increased relative to the control (except with Mazurka) and this was due mainly to an increase in pustule number and not individual pustule area except on Julia where there were increases in both. With periods between inoculations longer than 2 days both pustule number and size were reduced; the combined effect of which was to reduce the total pustule area per leaf. This effect was accentuated

TABLE 27A (See also Appendix Table 37) Dual infections of rust and mildew on four cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis or P.hordei respectively.

Days between Inoculations		Mean no. mildew colonies/leaf. 0	1	2	4	6
	Cultivar					
Leaves pre-inoculated with P.hordei	Midas	106	83	19	34	24
	Zephyr	122	76	17	8	0
	Julia	106	79	28	31	28
	Mazurka	-0	-0	-0	-0	-0
Leaves not pre-inoculated with P.hordei (Controls)	Midas	107	108	26	55	71
	Zephyr	106	82	30	43	106
	Julia	108	110	36	39	105
	Mazurka	-0	-0	-0	-0	-0

TABLE 27B (See also Appendix Table 38) Dual infections of rust and mildew on four cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis or P.hordei respectively.

Mean no. rust pustules/leaf.

Days between inoculations:	Cultivar	0	1	2	4	6
Leaves pre-inoculated with E.graminis	Midas	246	39	66	64	15
	Zephyr	201	94	58	112	0
	Julia	247	67	58	100	3
	Mazurka	162	128	52	97	25
Leaves not pre-inoculated with E.graminis (Controls)	Midas	249	27	51	144	106
	Zephyr	228	58	89	138	94
	Julia	220	96	145	154	132
	Mazurka	253	122	81	187	85

Table 28 A Dual infections of rust and mildew on four cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis and P.hordei respectively.

Mean area of mildew colonies (mm^2)

Days between inoculations:	Cultivar	0	1	2	4	6
Leaves pre-inoculated with P.hordei.	Midas	1.86	1.26	0.52	1.24	0.71
	Zephyr	1.92	3.13	1.68	1.28	1.05
	Julia	2.93	1.63	1.35	1.27	0.15
	Mazurka	-	-	-	-	-
Leaves not pre-inoculated (control)	Midas	4.29	2.28	3.65	4.86	3.27
	Zephyr	3.72	3.34	3.29	3.56	2.88
	Julia	3.57	3.18	3.90	3.83	3.18
	Mazurka	-	-	-	-	-

Table 28 B Dual infections of rust and mildew on four cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis and P.hordei respectively.

Mean area of rust pustules (mm^2)

Days between inoculations:	Cultivar	0	1	2	4	6
Leaves pre-inoculated with E.graminis	Midas	0.089	0.067	0.090	0.052	0.038
	Zephyr	0.085	0.096	0.075	0.053	0.058
	Julia	0.107	0.054	0.050	0.047	0.025
	Mazurka	0.076	0.063	0.066	0.058	0.058
Leaves not pre-inoculated with E.graminis (control)	Midas	0.122	0.092	0.114	0.074	0.092
	Zephyr	0.110	0.101	0.116	0.080	0.074
	Julia	0.085	0.078	0.134	0.074	0.069
	Mazurka	0.105	0.063	0.076	0.090	0.076

Table 29 (see also Appendix Table 39) Dual infections of rust and mildew on four cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis and P.hordei respectively.

Total area of mildew colonies per leaf(mm^2)

Days between inoculations:	Cultivar	0	1	2	4	6
Leaves pre-inoculated with P.hordei	Midas	197	105	10	42	17
	Zephyr	234	236	28	10	0
	Julia	311	129	37	39	4
Leaves not pre-inoculated with P.hordei	Midas	460	246	94	268	232
	Zephyr	395	272	97	153	305
	Julia	386	350	140	150	332

Total area of rust pustules per leaf(mm^2)

Leaves pre-inoculated with E.graminis	Midas	22	3	6	3	1
	Zephyr	17	9	4	6	0
	Julia	27	4	3	5	0
	Mazurka	12	8	3	6	1
Leaves not pre-inoculated with E.graminis	Midas	30	2	6	11	10
	Zephyr	25	6	10	11	7
	Julia	19	8	19	11	9
	Mazurka	27	8	6	17	6

as the period between inoculations were lengthened still further: Mazurka was the only cultivar not to support the growth of mildew and it is interesting to note that it was also the only cultivar where the total rust pustule area did not exceed that on the control. It is also noteworthy that the length of the period between inoculations at which total pustule area decreased (compared with the appropriate control) was related to the resistance of the cultivars to P.hordei: 1 day with Julia, the cultivar most resistant to P.hordei; 2 days with Zephyr and Mazurka, both equally susceptible to P.hordei and 4 days with Midas the cultivar most susceptible to P.hordei (and also fairly resistant to E.graminis).

When leaves were inoculated first with P.hordei and then with E.graminis, the effects on the total mildew colony area were mainly due to reductions in the colony size with short periods (0, 1 and 2 days) between the inoculations, and due to a combined effect on colony number and size where there were longer periods between the inoculations. The trends, with all cultivars where mildew could be recorded were similar. The total mildew area appeared to be reduced to a greater extent on Midas where there were short periods between the inoculations, and this was probably a consequence of the susceptibility to P.hordei of this particular cultivar. With all three cultivars the reduction in total colony area increased as the period between successive inoculations were lengthened and in these conditions there appeared to be little difference between the cultivars.

Fig 6 Leaves pre-inoculated with E.graminis

Total rust pustule area as % reduction from control.

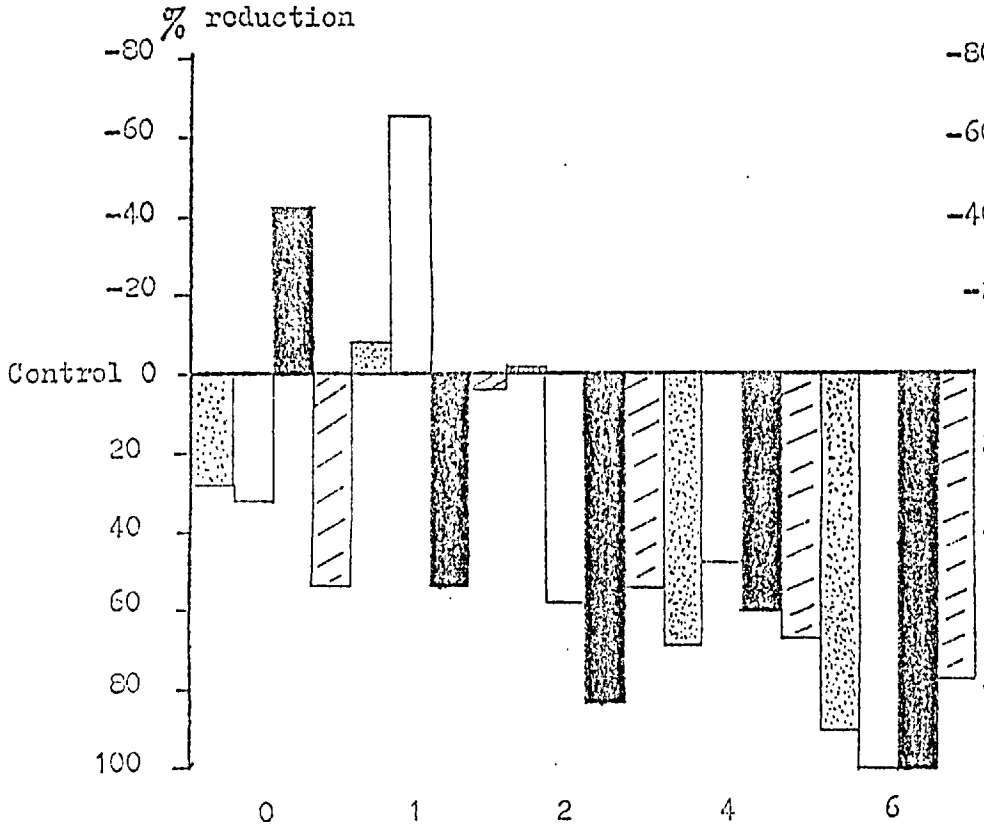
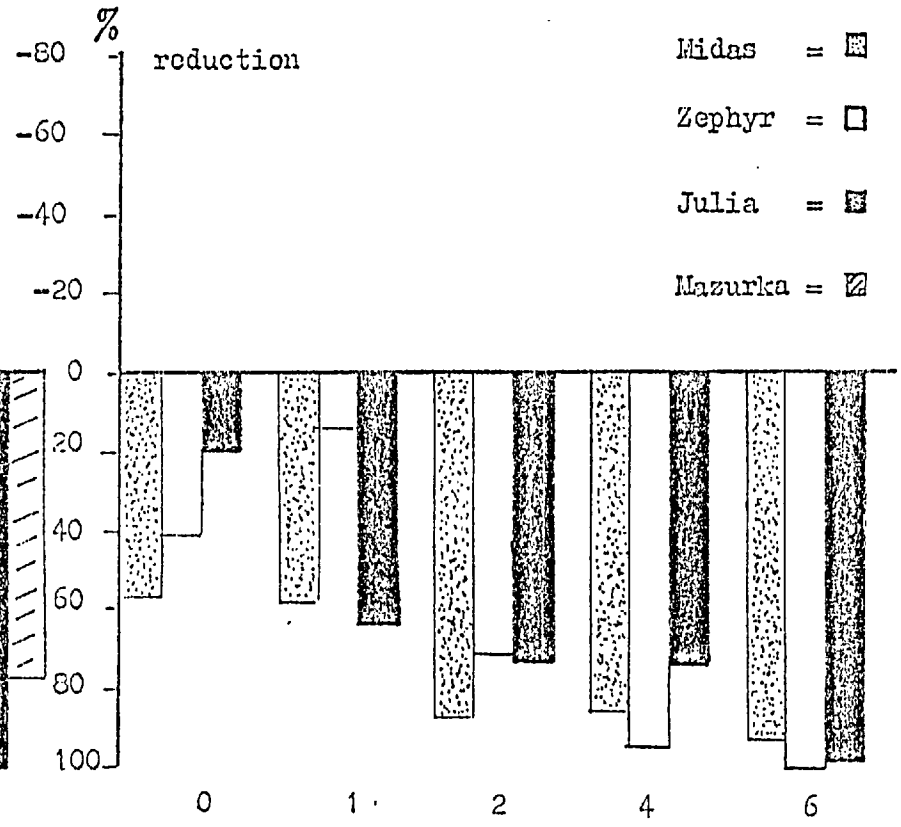


Fig 7 Leaves pre-inoculated with P.hordei.

Total mildew colony area as % reduction from control.



Dual inoculations of E.graminis and P.hordei on Mazurka and Zephyr.

Two situations were examined in further detail, they were:-

(A) Where the leaves were first inoculated with a barley pathogen but with a race to which the cultivar was resistant.

(B) Where the leaves were first inoculated with a non-pathogen.

In both situations the leaves were subsequently inoculated with virulent races of either P.hordei or E.graminis.

(A) Dual inoculations of E.graminis and P.hordei on Zephyr barley:

Conidia of E.graminis, race B 71/98 were dusted onto 10 day old barley seedlings in six pots. Four of these pots (two groups, A & B of two pots) contained seedlings of Zephyr, resistant to this race, and two pots contained the cultivar Proctor, susceptible to this race. Four other pots of Zephyr seedlings (groups C + D) were kept disease - free for 4 days after the inoculation. The seedlings in groups A and C (inoculated and control) were then inoculated with P.hordei and, similarly, groups B and D were inoculated with E.graminis race B 72/27. All plants were incubated appropriately and the number of mildew colonies and rust pustules was recorded 7 and 10 days respectively after the second inoculations. The amount of mildew on the two pots of Proctor seedlings was assessed after 10 days and was used to indicate the inoculum density of E.graminis B 71/98 on the Zephyr seedlings.

The results (Table 30) showed that pre-inoculating leaves with E.graminis race B 71/98 at an inoculum density which gave 80 - 90% cover on Proctor, reduced both the amount of rust and mildew (from race B 72/27) which developed from subsequent inoculations 4 days

Table 30 (see also Appendix Table 40) — Dual inoculations of E.graminis and P.hordei on Zephyr barley: (1) effects on mildew and rust of pre-inoculating leaves with a race of E.graminis to which Zephyr was resistant.

	Mean number of rust pustules per leaf.	Mean number of mildew colonies per leaf. (from inoculation with race B 72/27)
Leaves pre-inoculated with E.graminis, race B 71/98	135	5**
Leaves not pre-inoculated (control)	180	15

** Significantly different from control at $P \leq 0.01$ ('t' test)

later. However, only in respect of mildew was the difference from the control statistically significant.

Dual inoculations of E.graminis and P.hordei on Mazurka: (ii) effects on rust of pre-inoculating leaves with E.graminis.

E.graminis race B 71/98 caused no visible reaction on Zephyr but when Mazurka was inoculated with race B 72/27, brown necrotic flecks appeared after 4 days. The following experiment investigated the effect on rust development of this resistance reaction of Mazurka to E.graminis.

Eight pots of 10-day old barley seedlings were used, six of Mazurka and two of Zephyr. Three pots of Mazurka together with the two pots of Zephyr ('guide plants') were inoculated liberally with E.graminis B 72/27. One day later all six pots of the Mazurka seedlings were inoculated with P.hordei and incubated for 24 h. in a saturated atmosphere. After a further 9 days the number of rust pustules that developed on each leaf was recorded and the level of mildew on the Zephyr (guide) plants was assessed, (70 - 80% cover). The results detailed in appendix Table 41 show that the number of rust pustules was significantly reduced. ($P \leq 0.01$) (from a mean of 67 to 31 pustules per leaf) by pre-inoculating leaves with E.graminis.

(iii) Effects of pre-inoculating leaves with E.graminis at varying periods before inoculations with P.hordei.

This experiment and those which immediately follow, were

performed to characterize fully the effect seen in (ii) above. The methods used were similar to those described in Section 1 for the interactions on Zephyr barley.

There were eighteen pots of 10-day-old barley seedlings, sixteen of Mazurka and two of Zephyr. Eight pots of Mazurka (in groups 0A, 1A, 2A and 4A, each of two pots) and the two of Zephyr (guide plants – to indicate inoculum density) were liberally inoculated with E.graminis race B 72/27. The plants were then returned to filtered air cabinets together with the eight pots of uninoculated Mazurka (labelled in groups of two pots 0B, 1B, 2B and 4B).

On the same day two pots of the treated seedlings (0A) together with two pots of the control seedlings (0B) were inoculated with P.hordei. This procedure was repeated after 1, 2, and 4 days using groups of plants 1A, 2A, 4A with groups 1B, 2B and 4B to give the treatments shown in Table 31.

Mildew was assessed on the Zephyr barley after 10 days and the number of rust pustules per leaf on Mazurka 10 days after the appropriate inoculations with P.hordei.

The results showed that when Mazurka was inoculated with P.hordei almost immediately after (2 h) an inoculation with E.graminis the number of rust pustules was increased relative to the control, with longer periods between the two inoculations the reverse was true; there were fewer rust pustules though significantly so only at periods of 1 and 2 days.

Further investigations using Mazurka.

When seedling leaves of Mazurka were inoculated with E.graminis

TABLE 31 (See also Appendix Table 42) — Dual inoculations of P.hordei and E.graminis on Mazurka: effects of pre-inoculating leaves with E.graminis at different periods before inoculation with P.hordei.

Days between Inoculations	Mean no. rust pustules on leaves: Pre-inoculated with E.graminis (A)	not pre-inoculated (B)	% of control	Significance (t-tests)
0	255	183	139	P 0.05
1	70	117	60	P 0.05
2	82	155	53	P 0.05
4	48	82	58	N.S.

Mildew on Zephyr 'guide' plants was 100% cover after 10 days.

race B 72/27 very few colonies of any significant size were produced, but there were still substantial effects on the development of rust following a further inoculation with P.hordei. In this respect the results were similar to those obtained on Zephyr but without, apparently, involving a competition for nutrients or physical interference of germ-tubes from uredospores by the mildew colony.

These results encouraged further investigation into the nature of the interactions on Mazurka. There were three experiments. The first two examined the effects of using respectively, different surfaces of the same leaf and different leaves for the two inoculations. The third examined further the effects of one inoculation closely followed by another.

(1) Effect on rust of pre-inoculating different leaf surfaces of Mazurka with E.graminis.

Six pots of 10-day old Mazurka seedlings were divided into three groups of two (A,B and C). The seedlings in groups B and C were secured in the inoculating apparatus (Fig. 1) using rubber weights so that the adaxial leaf surfaces in group C were facing upwards. Two pots of Zephyr seedlings of similar age were also placed in the apparatus (as guide plants), and all plants were then inoculated with E.graminis. The uninoculated seedlings (group A) were kept disease-free for four days after this inoculation and then the Mazurka seedlings in all six pots were arranged with their adaxial leaf surfaces facing upwards and were inoculated with P.hordei. They were incubated in the standard way and the

number of rust pustules per leaf was recorded after 10 days, by clearing leaves in methanol and counting pustules over a light-box. Mildew on the Zephyr plants was assessed 10 days after their inoculation.

The results (Table 32) show that the development of rust on the adaxial surface was reduced to a similar extent where either this or the abaxial surface was pre-inoculated with E.graminis . This contrasted with the results on Zephyr (P.39) where the reduction was much greater on the adaxial surface which was pre-inoculated with E.graminis than on the abaxial surface which was not so treated.

(ii) Effect on rust on the second leaf of pre-inoculating the first leaf with E.graminis.

The seedlings in eight out of sixteen pots of 10 day old Mazurka were inoculated liberally with E.graminis so that many conidia were deposited on the first leaves. After 10 days, in which all pots of seedlings were kept under the same conditions, all plants were inoculated with P.hordei so that the second leaves received the inoculum. After a further 10 days (including 24 h. at high humidity) the number of rust pustules was recorded in a $2\frac{1}{2}$ cm section taken from the centre of each second leaf. The results (Appendix Table 44) indicated that there was no significant difference between the number of rust pustules which developed on the second leaves of plants whether the first leaves were pre-inoculated with E.graminis (mean 19 pustules per leaf) or not (mean 22 pustules per leaf)

Table 32 Effect on rust of pre-inoculating different leaf surfaces of Mazurka with E.graminis.

Mean number of rust pustules per leaf†		
Leaves not pre-inoculated with E.graminis(control)	Leaves pre-inoculated with E.graminis††	
	Adaxial surface	Abaxial surface
(A)	(B)	(C)
69.4	45.4*	40.3*

† Mean of nine to eleven leaves

††Mildew level on Zephyr guide plants was 80 to 90 % cover after 10 days.

* Significantly different from the control at $P \leq 0.05$ ('t'test); value for B not significantly different from that of C

(iii) Inoculation of first leaves of Mazurka with E.graminis, followed immediately by inoculation with P.hordei.

Three pots of 10-day old Mazurka seedlings, plus one pot of Zephyr seedlings were inoculated with E.graminis, after 2 h the inoculated Mazurka seedlings, together with three pots of hitherto untreated seedlings were inoculated with P.hordei. All plants were then incubated in the usual way and after 10 days the rust pustules were counted. For this the leaves were detached, cleared in methanol and the number of rust pustules recorded in ten microscopic fields chosen at random from the centre of each leaf. This procedure was adopted because the necrotic flecks which developed following inoculation with E.graminis interfered with the assessment of rust by eye.

The results (Appendix Table 45) indicate that there was an increase in the number of rust pustules on leaves pre-inoculated with E.graminis, but that this was not sufficiently large to be different statistically from the control.

Dual inoculations of E.graminis and P.hordei on Mazurka: causes of the effects observed.

(i) Effects on P.hordei of pre-inoculating leaves with E.graminis: Area of necrotic flecks and effects on the width of stomatal apertures.

This experiment was performed simultaneously with that described in Section 1 Page 58 where similar features were recorded for Zephyr barley inoculated with E.graminis. The only alteration in the procedure was that the area of the necrotic flecks instead of mildew colonies was determined, the aim being to see if the area of leaf made unavailable to P.hordei was large enough to account for the observed reduction in rust. The results (Table 33) show that the areas of the necrotic flecks, produced as a consequence of inoculation with E.graminis were at no time sufficiently large to account for the 55% reduction in numbers of rust pustules. The width of the stomatal apertures on inoculated leaves was the same as on the control leaves at the times and conditions of measurement.

(ii) Effects on rust development of pre-inoculating leaves with different amounts of E.graminis.

The seedlings in six pots of Mazurka and four pots of Zephyr barley were inoculated with conidia of E.graminis using five mildewed leaves. After allowing conidia to settle one pot each of Mazurka and Zephyr seedlings were removed from the inoculation chamber, and the seedlings in the remaining pots were re-inoculated with conidia from five other leaves of the same batch of infector plants. Again after the conidia had settled one pot each of

Table 33 (see also Appendix table 46) — Inoculation of leaves with E.graminis: area of the necrotic flecks and effects on the width of stomatal appertures.

	Days from inoculation with E.graminis			
	4	6	8	12
Total area of necrotic flecks per leaf(mm^2)	1.67	8.70	13.75	17.96
Mean width of stomatal apertures(μ)				
Uninoculated leaves	1.03	0.91	0.82	1.19
Inoculated leaves	1.05	0.84	0.86	1.06

Mazurka and Zephyr seedlings were removed. This re-inoculation procedure was repeated twice more using respectively ten and twenty leaves from the infector plants, and after each inoculation two pots of seedlings were removed to give the treatments A – D in Table 34. After 4 days the Mazurka seedlings together with three pots of untreated (control) seedlings were inoculated with P.hordei. They were incubated as usual and returned to a filtered-air cabinet, where after 2 and 4 days, five leaves were taken from the excess of treatment D and control plants. Impressions (using Sellotape) were made of these leaves sampled after 2 days and after staining these were used to assess the percentage germination of the uredospores. Those leaves sampled after 4 days were cleared and stained in lactophenol- cotton blue; They were then recleared in plain lactophenol and examined microscopically. The uredospores that had germinated and whose germ tubes had entered stomata were counted and the presence or absence of primary infection sites was noted in each case.

The mildew levels were assessed on the Zephyr 'guide' plants 10 days after inoculation with E.graminis. On Mazurka the number of rust pustules was recorded 10 days after inoculation with P.hordei on those leaves remaining in treatment D and on the control plants, as well as on those in treatments A, B and C.

The results (Table 34) showed that the number of rust pustules per leaf decreased as the inoculum levels of E.graminis increased. The reduction in the number of rust pustules in treatment D resulted from fewer primary infections being established by germinating uredospores.

Table 34 (see also Appendix table 47) Effects on rust development of pre-inoculating leaves with different amounts of E.graminis

Leaves inoculated with E.graminis

Number of infector leaves used		Percentage cover of mildew on Zephyr after 10 days.	Mean no. rust pustules per leaf. Mazurka pre-inoculated with E.graminis	Percentage of control rust level
A	5	15	74	68
B	10	30	62	57
C	20	60	58***	54
D	40	90	43**	40
Control	0	0	108	100

	Mean % uredospore germination	Number of uredospore germ tubes reaching stomata		
		total	with infection sites: present	absent
Control	38	26	20	6
Treatment D	38	29	13*	16

Values significantly different from the control at $P \leq 0.05$; 0.01; and 0.001 denoted by *, **, and *** respectively.

(iii) Effects of materials likely to alter the carbohydrate content of leaves.

The pre-inoculation of Mazurka seedlings with E.graminis reduced the subsequent development of rust. It was considered that similar effects on Zephyr barley (P 60) might result in part from a competition for nutrients between the two pathogens. Although at first sight this seems less likely to be true for the effects observed on Mazurka, since E.graminis (race 72/27) produces no more than necrotic flecks on this cultivar, it was investigated in two experiments similar to those performed with Zephyr which aimed to alter carbohydrate content of leaves.

(a) Floating leaf segments on sucrose.

This experiment was performed at the same time, and using the same procedure as that with Zephyr barley described in Section 1 (P. 67).

The results with Mazurka (Table 35) showed that the reduction in rust pustule size observed on leaves pre-inoculated with E.graminis could partly be reversed by an exogenous supply of sucrose. The results also showed that the sucrose treatments caused a reduction in the chlorophyll content of leaf segments, an effect apparently opposite to the effect of inoculating the leaf segments with E.graminis.

(b) Treating plants with maleic hydrazide.

Eight pots of 10-day-old Mazurka seedlings were used in this experiment and these were divided into four groups, A and C each with one pot only and B and D each with three pots (see Table 36). There were two inoculations. In the first the seedlings in group

Table 35 (see also Appendix table 48) — Effect on rust of pre-inoculating leaves with E.graminis and floating them on 2 % sucrose.

	Leaves not pre-inoculated with E.graminis, placed on:		Leaves pre-inoculated with E.graminis, placed on:	
	water (A)	2% sucrose (B)	water (C)	2% sucrose (D)
Mean area of rust pustules (mm^2)	0.209	0.228	0.120*	0.175†
Total chlorophyll mg/litre	1.89	1.02	3.20	1.72

* significantly different from control(A) at $P \leq 0.05$('t' test)

† significantly different from (C) at $P \leq 0.05$

C and D were inoculated with E.graminis, and in the second, 4 days later, the seedlings in all groups were inoculated with P.hordei. Plants in B and D were treated with maleic hydrazide daily as a root drench (50ml of a 0.1 % solution per pot) beginning 1 day after inoculation with E.graminis and continuing until the number of rust pustules was counted 10 days after inoculation with P.hordei. The areas of rust pustules were also determined at this stage by clearing the leaves in methanol and measuring pustule dimensions microscopically.

The results of this experiment (Table 36) showed that treatment with maleic hydrazide of leaves pre-inoculated with E.graminis did not reverse the apparent effect of reducing the size of the rust pustules. The number of rust pustules appeared to be reduced by both the treatment with maleic hydrazide (treatment B) and the pre-inoculation of leaves with E.graminis (treatment c), but in neither case was the reduction significant. However the recorded number of pustules on plants in treatment D (both maleic hydrazide and pre-inoculation of leaves with E.graminis.) was greater than would have been expected from an accumulation of the apparent reductive effects of the two treatments (B and C).

(iv) Inoculation of leaves with E.graminis: effects on the chlorophyll content and dry weight of leaves.

Although E.graminis (race B72/27) fails to develop on Mazurka it obviously has effects on the leaf, some of which may concern its nutritional status as indicated by parts of the previous experiments. The effect of E.graminis on the chlorophyll content and dry weight of Mazurka leaves are therefore examined.

Table 36 Effect on rust of pre-inoculating leaves with E.graminis and treating them with maleic hydrazide.

	Leaves not pre-inoculated		Leaves pre-inoculated	
	Control (A)	Maleic hydrazide. (B)	Control (C)	Maleic hydrazide. (D)
Mean no. rust pustules per leaf	43.6	34.4	29.1	29.1
Mean area of rust pustules (mm^2)	0.079	0.100**	0.061	0.059

** significantly different from control at $P \leq 0.01$ ('t' test)

Thirty pots of Mazurka seedlings were grown to the first leaf stage under standard conditions, half of the seedlings were inoculated with E.graminis race B 72/27 and the other half kept disease - free as controls. Two pots of Zephyr which received the same inoculation were used as the usual guide to inoculum density. After the inoculation, and at the same time of day on every third day for 24 days and then every fifth day, until the leaves completely senesced, ten leaves were sampled at random from each of the two sets of plants.

Each batch of ten leaves was treated as follows: four leaves (two replicates of two leaves) were weighed, dried for 3 days at 70 C, and reweighed for dry weight determination; and six leaves (two replicates of three leaves) were weighed, macerated in methanol (10 mls per replicate) in a Sorvall Omnimixer, and filtered. The diluted filtrates were used to determine the chlorophyll concentrations by the spectrophotometric method of McKinney, (1941).

Four days after the initial inoculation with E.graminis, two pots of seedlings from the inoculated and uninoculated plants were inoculated with P.hordei. The plants were incubated and the number and size of the rust pustules that resulted were determined after 10 days.

The mildew that developed on the Zephyr 'guide' plants 10 days after inoculation was assessed to be 80 - 90% cover.

The results (Table 37) indicated that although mildew did not develop on Mazurka the inoculation with conidia resulted in an accelerated chlorophyll loss. The dry weight to fresh weight ratio was also increased following inoculation with E.graminis. The

TABLE 37 (See also Appendix Table 50) Inoculation of leaves with E.graminis: effect on the chlorophyl content and dry weight.

Days after inoculation.	%dry weight: Control leaves	%dry weight: Inoculated leaves	mg/chlorophyl per g. dry wt.: Control leaves	mg/chlorophyl per g. dry wt.: Inoculated leaves
0	7.405	7.25	21.06	19.89
3	7.30	7.50	22.23	19.04
6	7.03	7.80	22.84	16.09
9	6.58	8.25	23.18	14.73
12	6.65	8.25	21.64	13.62
15	6.35	8.90	21.02	10.37
18	6.60	8.80	15.42	8.83
21	7.30	14.10	12.83	2.93
24	6.80	24.90	13.34	2.42
29	7.80	90.7	9.55	2.09
34	33.50	-	0.79	-

Leaves inoculated with P.hordei.

	Control leaves: not pre-inoculated.	Leaves pre-inoculated with E.graminis.
Mean no. rust Pustules/leaf	75.2	41.5**
Mean area rust pustules(mm^2)	0.066	0.044***

Significant difference (by 't' tests) from control leaves at $P \leq 0.002$

numbers and areas of rust pustules were also significantly reduced on leaves which were inoculated with P.hordei 4 days after the treatment with E.graminis.

(v) Inoculation of Mazurka with E.graminis: effect on antifungal compounds.

Inoculation of E.graminis race B 72/27 onto leaves of Mazurka (a) invoked resistance reactions that were visible 4 days after the inoculation, and (b) caused the leaves considerable damage which could be detected by the effects on the chlorophyll content and percentage dry weight 6 or more days after inoculation. These effects could account for some of the observed reductions in rust pustules in dual inoculations especially where 4 days or more elapsed between the first inoculations with E.graminis and the second with P.hordei. With intervals less than this their involvement appears less clear and in these instances antifungal compounds might be more important. The possible role of such materials was thus examined using the two extraction procedures detailed in Section 1 (P. 83) and similar spore germination tests on these extracts to assess their antifungal activity (P 85).

(A) The first extraction procedure was based on that of Stoessl and Unwin, (1970) for the 'Hordatine' group of compounds. Two 25g. leaf samples were extracted; one of leaves inoculated 4 days previously with E.graminis and the other of untreated leaves of comparable age. The U.V. absorbing properties of the extracts were compared for their U.V. absorbing properties, and spore germination tests were performed (Table 38). The remaining extracts

Table 38 (see also Appendix Table 51) Inoculation of Mazurka with E.graminis: effects of antifungal compounds - spore germination tests. (Extraction method 1)

Mean percentage germination[a]

Dilution	Conidia A	Conidia B	Uredospores A	Uredospores B
0[b]	22	17	3.5	3.8[c]
10^{-1}	29	22	7.3	8.0
10^{-2}	30	26	23.7	16.7*
10^{-3}	33	28	26.8	22.0
Distilled water controls	39,34.		28,33.	

[a] Mean % germination based on minimum of 650 conidia or uredospores.

[b] Dilution 0 = approximate tissue concentration

[c] A = extract from uninoculated leaves

B = extract from leaves inoculated with E.graminis

* Significantly different ($P \leq 0.05$) from corresponding value in A

were sprayed (with tween 80 as a wetting agent) onto 10-day-old Zephyr barley seedlings. Similar seedlings were sprayed with tween 80 solution only as controls, and seedlings from both groups of plants were inoculated with either E.graminis or P.hordei. The number of mildew colonies and rust pustules per leaf were recorded 7 and 10 days after the appropriate inoculations, and the size of the mildew colonies and rust pustules were measured from a random sample taken from the central 5 cm. portion of each leaf (Table 39).

Spectrophotometry showed three similar peaks in the absorbance of both extracts at 210 -220, 260 -270 and a broad band around 300-330nm, the difference between extracts being only in the amount of absorbence at these wave lengths. That the extract from inoculated leaves differed little from that obtained from the control leaves was confirmed by tests of antifungal activity using conidia of E.graminis and uredospores of P.hordei (Table 38). Only in one instance was germination significantly less with the extract from inoculated leaves (10^{-2} dilution using uredospores).

When leaves of Zephyr were sprayed with either of the extracts before inoculating them with E.graminis, the number and size of the mildew colonies which developed were less than on the corresponding controls (Tables 39) but there was no difference in these respects between the two extracts. The extracts had no effect on rust development when applied to leaves before they were inoculated with P.hordei.

(B) The second extraction procedure was similar to that described in Section 1 Page 84 except that it was terminated after the separation into ether and agueous phases. The starting material

Table 39 Inoculation of Mazurka with E.graminis: effect of antifungal compounds - tests of extracts against infection of Zephyr by the two pathogens.

Extract used to spray leaves.	Mean no. and area(mm^2) of colonies/pustules[a]			
	E.graminis		P.hordei	
	Number	Area	Number	Area
Nil-water + Tween 80 (control)	135	2.26	76	0.076
10^{-1} dilution				
uninoculated leaves	86*	1.72*	46	0.074
inoculated leaves	81**	1.78*	68	0.079
10^{-2} dilution				
uninoculated leaves	-	-	59	0.077
inoculated leaves	-	-	69	0.080

[a] Mean no. based on counts of ten leaves and area on measurements of 80 colonies or pustules.

Values significantly different from the corresponding control at $P \leq 0.05$ and 0.01 denoted by * and ** respectively.

was 8 g fresh weight of leaves inoculated 4 days previously with E.graminis and a similar quantity of leaves not so inoculated (control). The antifungal properties of the two fractions from each lot of tissue was subsequently tested using conidia of E.graminis and uredospores of P.hordei. For this, the fractions were first evaporated to dryness under vacuum and redissolved in 8 ml. of distilled water to give approximate tissue concentration. The results are shown in Table 40 and in further detail in Appendix Table 53.

The germination of conidia was inhibited by both fractions derived from the inoculated leaves, and by the ether fraction from control leaves. Uredospore germination was inhibited by all fractions, but those from the inoculated leaves showed greater inhibitory activity than those from control leaves.

These results indicate that the extracts from uninoculated Mazurka seedlings contained spore germination inhibitors. The amounts of these appeared to be greater in extracts of inoculated leaves.

Table 40 Inoculation of Mazurka with E.graminis: effect of anti-fungal compounds - spore germination tests

(Extraction method 2)

Fraction tested:	Mean percentage germination [a] Conidia A	Conidia B[c]	Uredospores A	Uredospores B
Aqueous[b] fraction	32.3	12.46**	6.1	0.7**
Ether fraction	7.4	11.7	9.2	0.2**
Distilled water control	38.7		27.2	

[a] Mean % germination based on minimum of 500 conidia or uredospores

[b] Approximate tissue concentration

[c] A = extract from uninoculated leaves

B = extract from leaves inoculated with E.graminis

** Values significantly different ($P \leq 0.05$) from corresponding value in A

Dual inoculations on Zephyr: (B) effects on rust and mildew of pre-inoculating leaves with Diplocarpon rosae or Uromyces phaseoli.

Two experiments were performed. In the first, four pots of Zephyr barley seedlings (group A and B) were inoculated with uredospores of Uromyces phaseoli using a powder dispenser. These were incubated in a saturated atmosphere for 24 h with two pots of uninoculated control seedlings (labelled C and D). After a further 3 days three pots of seedlings, i.e. two pots of inoculated seedlings group A, and one pot of control seedlings group C, were inoculated with P.hordei. Similarly the remaining three pots of seedlings, groups B and D, were inoculated with E.graminis. The seedlings were incubated and 10 days after these inoculations the number of rust pustules and percentage cover of mildew were assessed.

In the second experiment the same procedure was pursued except that Diplocarpon rosae was inoculated onto leaves in place of Uromyces phaseoli. The results are shown in Appendix Table 54. They indicate that pre-inoculation of seedling leaves of Zephyr barley with either Uromyces phaseoli or Diplocarpon rosae had little effect on the development of rust or mildew. A reduction in the amount of rust was observed when leaves were pre-inoculated with Uromyces phaseoli but the difference from the control was not statistically significant.

SECTION 3.

The effects of BAS 3170F and ethirimol on E.graminis and P.hordei respectively and on yield.

Introduction.

In the field trials conducted by Simkin (1971 and 1972) as well as those described for 1974 and 1975 (Section 4) four disease situations were obtained by the use of the two specific fungicides, Calirus and ethirimol. Ethirimol used either as a seed dressing (Milstem) or spray treatment (Milgo) controlled E.graminis and this allowed the development and study of P.hordei on its own as well as its effect on yield. Similarly spray treatments of BAS 3170F (Calirus) which controlled P.hordei, allowed an examination of the development of E.graminis and its effect on yield. To do this it was necessary to assume that the BAS 3170F and ethirimol treatments were not directly affecting E.graminis and P.hordei respectively and also that they themselves did not directly affect the yield.

The experiments described here were designed to test these assumptions.

Materials and Methods.

All plants were grown under the same conditions as previously described in Section 1 and 2. E.graminis race B 72/27 and P.hordei race Pb60/3/1 were used and all conditions of inoculation and incubation were as before. Where necessary a Weyco Climatic Cabinet Plant Growth Type 250 PG was used.

BAS 3170F was obtained from BAS F Ltd. and, ethirimol seed

dressing and spray from Plant Protection Ltd. Both fungicide sprays were used at the advised 'field rate' concentration using a Shandon Chromatography spray and seed dressed with Milstem were either obtained commercially from Elsons of Spalding Ltd., or were dressed at Plant Protection Ltd., Jealotts Hill, using a manually operated rotating drum.

The methods of assessment of the productivity of the two pathogens are described under the appropriate experiments.

Experimental.

(1) The effects of BAS 3170F on the development of E.graminis.

The first leaves of 10-day-old Zephyr seedlings contained in six pots were uniformly inoculated with E.graminis, race B 72/27. The plants were then placed in filtered-air-cabinets where the mildew was allowed to develop for 4 days. The plants were then divided into three groups and treated in the following ways:

Group 1 was sprayed to run-off with water only as a control.

Group 2 was sprayed to run-off with BAS 3170F (0.01% 75% w.p.) and citowett (0.0025%).

Group 3 was sprayed to run-off with citowett only (0.0025%).

The plants were left for 2 days during which time the mildew developed into easily visible colonies. Five leaves were then detached from each treatment and each group of leaves were used as a source of inoculum (in the apparatus shown on page 12) to inoculate separately three lots of three pots of disease-free Zephyr seedlings with E.graminis. After inoculation the leaves were recovered from the inoculum carrier and the number of mildew colonies per leaf and their size were determined microscopically.

A second sample of leaves was taken from each of these three groups 11 days after they had been inoculated with E.graminis and these, too, were used as sources of inoculum for the inoculation of more disease-free plants, using a similar procedure. The only difference was that 24 h before they were required as a source of inoculum the leaves were shaken to remove old conidia.

The plants which were inoculated in this way on the sixth, and eleventh days of the experiment (secondary plants) were returned to the greenhouse after inoculation and the number of colonies that developed was recorded 7 days later. The results of this experiment are shown in Table 41 and in greater detail in Appendix Table 55. They show that while applications of Calirus and Citowett or of Citowett alone did not affect the number of successful infections they did severely affect the growth of the mildew, colonies on these treated plants being significantly smaller than on the controls. As a result of this, the productivity of colonies was affected, as was shown by the different amounts of mildew which developed on the 'secondary plants'. Lack of any significant difference in these respects between plants in group 2 (Calirus and Citowett) and those in group 3 (Citowett only) indicated that the effects observed were due to the wetting agent and not Calirus.

(11) The effect of ethirimol on the development of P.hordei.

Three pots were sown with Zephyr seed treated with Milstem and three with seed not so treated. Ten days after sowing the seedlings were uniformly inoculated with uredospores of P.hordei. They were incubated, and after 10 days the number of rust pustules that had

Table 41 The effects of Calirus on the development of E.graminis.

	Treatments		
	1.Water only (control)	2.Calirus:+ Citowett	3.Citowett only
Mildew development on treated plants			
6 days after inoculation			
mean no.colonies per leaf	70	81	82
mean area of colonies(mm^2)	3.154	0.604^{**} -ns-	0.595^{**}
11 days after inoculation			
mean no.colonies per leaf	60	53	60
mean area of colonies(mm^2)	8.679	$3,661^{**}$ -ns-	3.208^{**}
Mildew development on'secondary' plants (as mean no.colonies per leaf) using treated leaves as sources of inoculum after:			
6 days	35	20^{**} -ns-	23^{**}
11 days	18	12^{**} -ns-	10^{**}

Values significantly different from the control at $P \leq 0.05$ and 0.01 denoted by * and ** respectively, values not significantly different (on the same line) denoted by -ns-

developed was recorded for each leaf.

The results, (Appendix Table 56) showed that there was no significant difference in the susceptibility to P.hordei of seedling leaves of plants grown from Milstem-dressed or from those seeds not so treated.

However there was some doubt as to whether or not first seedling leaves would have taken up sufficient ethirimol to have had any effect on the rust. Consequently a second experiment was performed using the third leaf of Zephyr barley and including a Milgo spray treatment.

Six pots of barley were sown, three pots contained Milstem-dressed seed, and the remainder contained normal single-purpose mercury dressed seed. The plants were maintained disease free in filtered air cabinets until the third leaf was fully expanded. At this stage the seedlings in the three pots containing the Milstem-dressed seed were sprayed with 0.01% 'Milgo'. On the following day all six pots of plants were uniformly inoculated with P.hordei by the usual procedure. After the standard incubation the plants were returned to filtered air cabinets and kept for 20 days. The leaves were then detached and 5cm. sections taken from each leaf; these were cleared, stained and examined microscopically. The number of rust pustules per unit area was assessed by counting the number of pustules in five random microscopic fields (x 28 magnification). The area of the rust pustules was determined at the same time . The results, (Appendix Table 57) showed that the Milstem seed dressing and Milgo spray had no significant effect on the number or size of the rust pustules that developed.

The effect of ethirimol on the pustule productivity of rust.

Ethirimol has been reported to delay senescence (Finney, 1973) and the effect of this delayed senescence on the duration and productivity of rust pustules was examined in the following experiments.

There were three groups of plants each with three pots of 10-day-old Zephyr barley. The first group was not chemically treated (control); the second had Milstem-dressed seeds, and the third was sprayed with Milgo (0.01%) on the tenth day after sowing.

On the eleventh day all plants were uniformly inoculated with P.hordei, incubated as usual and then placed in a growth cabinet (Weyco Environmental Cabinet) at 20°C with a 16 h photo period.

After 10 days the number of pustules was recorded on each leaf, and the uredospores from each of the three pots of plants in each group were collected using nine weighed cyclone collectors. These collections were weighed. Similar collections were taken every 2 days for 10 days at which time the leaves were senescent.

The results (Appendix Table 58) indicated that there were no significant differences between the weights of spores collected from plants in each treatment. The collection technique was however, less than satisfactory, the loss of uredospores during collection, the damage to the leaf and the collection of fragments of senescent leaf tips all contributing to the variability in the results.

The experiment was therefore repeated using Milstem-dressed and undressed seed as the only treatments. Leaves inoculated with P.hordei were enclosed in open ended glass tubes (Johnson and Bowyer,

1974) and plants were kept in a filtered-air cabinet which was less draughty than the growth chambers. The leaves were left inside the tubes until they completely senesced (30 days from inoculation). The uredospores were then collected in the dry state from each tube, and weighed accurately. The senescent leaves were soaked in 50% glycerine to enable the number of rust pustules per leaf to be recorded.

The results are shown in Table 42 and Appendix Table 59. These appeared to show that the rust pustules on first leaves from Milstem dressed seed produced more uredospores overall than those on leaves from undressed seed; but the differences were not sufficiently large to be statistically significant.

TABLE 42 (See also Appendix Table 59) — The effect of ethirimol on the pustule productivity of rust.

	P.hordei inoculated onto plants grown from:		
	non-milstem dressed seed (control)		milstem dressed seed
Mean no. pustules/leaf	112.6		108.4
Mean wt. of uredospores/pustule (mg)	0.015	- n.s. -	0.021

The effects of BAS 3170F and ethirimol on barley yield in the absence of P.hordei and E.graminis.

The assessment of yield losses due to mildew and brown rust is difficult because in the field disease-free plants for comparison can only be obtained by using chemicals and these could affect yield directly as well as through their fungicidal action.

The following experiment was performed to determine the effect BAS 3170F and ethirimol have on the yield of Zephyr barley in the absence of disease by using filtered-air cabinets in the greenhouse.

Sixteen pots (8in., 17 cm.) containing field soil were sown with Milstem-dressed seed in March 1975, three seeds per pot (treatment 1). Thirty-two similar pots were sown with seed treated only with a standard mercury dressing (treatments 2 and 3). All pots were then placed at random in two filtered-air cabinets inside a greenhouse on a self-watering sand bench. The temperature throughout this experiment was kept as near to that outside as possible so as to provide normal growth and tillering. When seedlings emerged they were thinned to two per pot.

Two months after sowing the sixteen pots of plants in treatment 1 each received a root drench of ethirimol (100 ml. 0.01% as the hydrochloride). Sixteen pots labelled treatment 2 received a spray treatment of BAS 3170F (0.01, 75% wp. in 0.0025% Citowett). The seedlings in the remaining sixteen pots (treatment 3 control) together with those in treatment 2 were given a control root drench of 100ml. water.

After a further 2 months, when the grain had ripened the number of ears per plant was recorded. They were then harvested by

hand, dried at 70°C for 4 days and weighed. The results (Appendix Table 60) demonstrated that neither ethirimol nor BAS 3170F at the concentrations and under the conditions used had any effect on the yield of Zephyr barley.

4. Interactions between P.hordei and E.graminis on Field Grown Barley.

A. Field Trial 1974. Silwood Park.

Introduction.

The previous experiments have described the interactions between P.hordei and E.graminis following inoculations of the same barley leaf. The extent and nature of these interactions are themselves intrinsically interesting but the questions arise whether such interactions occur extensively in barley crops and, if so, how they affect overall yield. The field trials conducted in 1971 and 1972 by Simkin with the cultivar Zephyr sought answers to these questions but relatively few indications of mildew x rust interactions were obtained. Thus in these trials there were no significant interactions in respect of yield or senescence of the leaves though in one trial the infection rate of E.graminis was higher on leaves without rust indicating that the presence of rust pustules significantly affected the development of mildew on the same leaf(Simkin & Wheeler, 1974b). The primary aim of the present trial was to re-examine interactions in the field, hopefully in a situation where brown rust was more severe than the 5% level in Simkin's trials and with cultivars of different suscepibilities to P.hordei.

Materials and Methods.

The trial, of randomized block design, was set up at Silwood Park on a light, sandy, well-drained soil previously cropped to potatoes. Three cultivars - Zephyr, Midas and Julia - were each subjected to four treatments. These were: (1) Mildew allowed to develop, rust controlled; (2) Rust allowed to develop, mildew controlled; (3) Mildew and rust allowed to develop; (4) Mildew and rust controlled. Each cultivar- treatment combination was replicated three times on plots 6 x 15 ft (2 x 5 m). These plots were sown on 8 April, but to encourage the development of brown rust within the site strips of the most susceptible cultivar, Midas, were planted earlier, on 26 March, between adjacent plots in each block as indicated in Fig 8 .

Mildew was controlled, in treatments (2) and (4) and within the Midas strips by sowing Milstem-dressed seed and subsequently, on 12 June, by spraying with Milgo(Plant Protection Ltd.) at the rate of 1.41/ha. Rust was controlled in treatments (1) and (3) by spraying, on 1 and 11 July, with Calirus (75% w.p.; BASF) at the rate of 2.78 Kg/ha.

Weeds were removed by hoeing between the rows until the crop was well enough established to overshadow the remaining weeds. At this stage (Growth stage 6; Large,1954) six bamboo canes were used to mark the sites of six randomly-selected plants in each of the plots of treatments 1, 2, and 3. The plants were selected from within the plots rather than at the edges, and were themselves labelled with Jeweller's tags during and after stem extension.

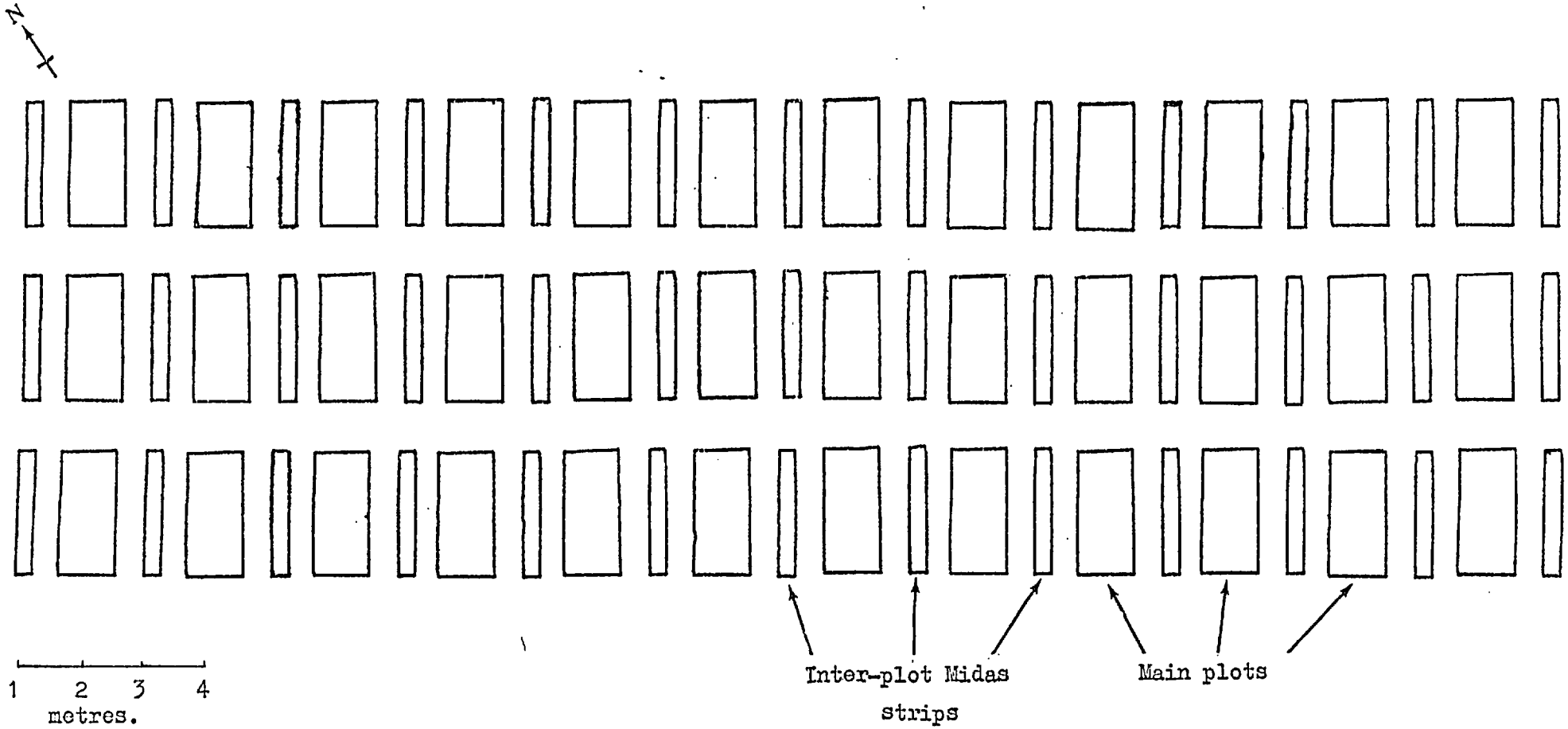


Fig 8. Layout of field Trial. 1974.

Disease was assessed on individual leaves(upper and lower surfaces) of marked plants using the standard diagrams of the Plant Pathology Laboratory, Harpendon, at weekly intervals from 4 June to 16 July, and then twice weekly until 26 July.

On 21 July a random sample of ten leaves immediately below the flag leaf (ie.leaf 2, flag leaf = 1) was taken from each plot of treatments 2 (rust only) and 3 (rust and mildew). Lengths(20 cm) were cut from the centre of each leaf and these leaf segments were then cleared in methanol, stained in lactophenol-cotton blue and the number and size of rust pustules were recorded microscopically.

On 8 August, when most of the crop was dry and ready for harvesting, five late tillers were selected at random from each plot and the disease levels assessed on their flag leaves.

The crop was harvested on 16 August. Each individual plot was cut by hand and transfered to a threshing machine(Dania, model T57279) which was tractor-driven, using a belt attachment.

The weights of grain for each plot were recorded. The percentage moisture content of the grain was calculated by drying 200g samples from each plot for 3 days at 70°C and then reweighing these. The weight of grain per plot was adjusted accordingly.

The dried grain was used in sieve tests. Samples (100g) were placed in the top of a stack of three sieves,(2.8, 2.5 and 2.2 mm mesh). The sieves were secured firmly to a mechanical shaker which was operated for 3 min. The grain was then collected from each sieve and weighed.

Dried grain was also used to determine 1,000 grain weights for each plot. Three replicate samples of 100 grains were weighed and the mean values obtained were multiplied by ten.

Results

1. Crop development and weather

The meteorological data for the 1974 growing season, obtained from Silwood Main Meteorological Site Latitude 51° 28'N are shown in Fig 9.

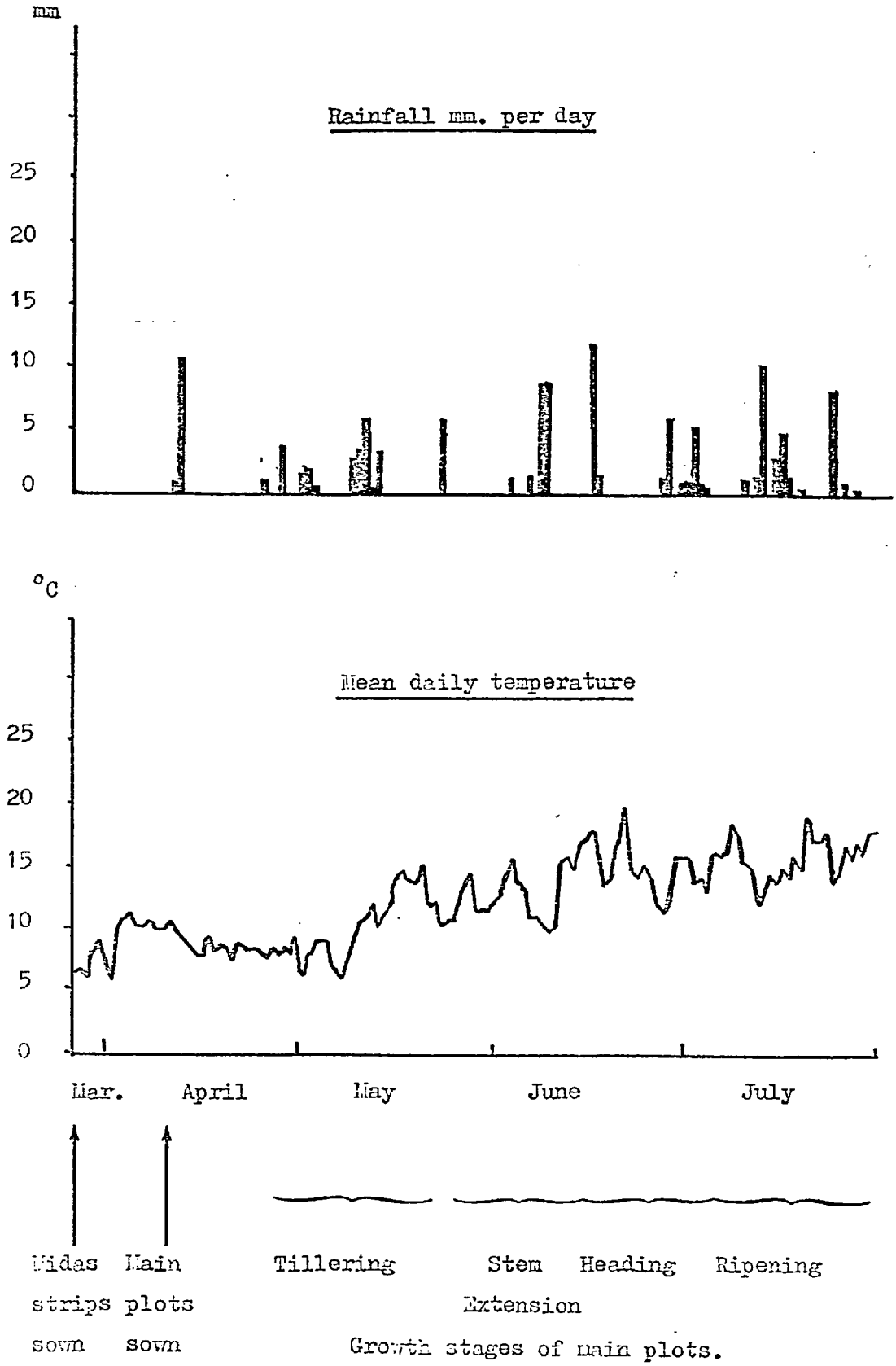
The Midas inter-plot strips were sown immediately prior to a period of warm, very dry weather which led to poor germination and growth. The plots themselves were sown just before 2 days of heavy rain, and consequently the barley from the two sowings developed at similar rates.

In late April and early May there was a short period of rainy weather with temperatures $\underline{c}$ 10° - 15°C. Mildew was first observed in the field on 12 May and on 11 June, after a period of warmer weather and intermittant rain, brown rust was first detected.

June was a month of average rainfall and temperature, and for the remainder of the growing season rain was frequent with temperatures averaging $\underline{c}$ 15°C. These conditions led to mildew levels as high as 50 to 60 % cover on untreated leaves, and to rust levels on susceptible untreated leaves as high as 20 %. These conditions also delayed ripening and heavy rain during the first week of August delayed harvesting until 16 August.

Figure 9 Meteorological Data Silwood Park 1974.

Latitude 51° 28' N



2. Interactions.

The mean percentage cover per leaf of rust and mildew for each assessment date plotted against time (Fig 10) summarizes the development of rust and mildew on the three cultivars both singly (treatments 1 and 2 for mildew and rust respectively) and in combination (treatment 3).

It would appear from this figure that whereas mildew developed similarly whether leaves were rusted or not, rust developed differently in the presence of mildew. The development of the two diseases is now considered in greater detail.

a. Development of mildew.

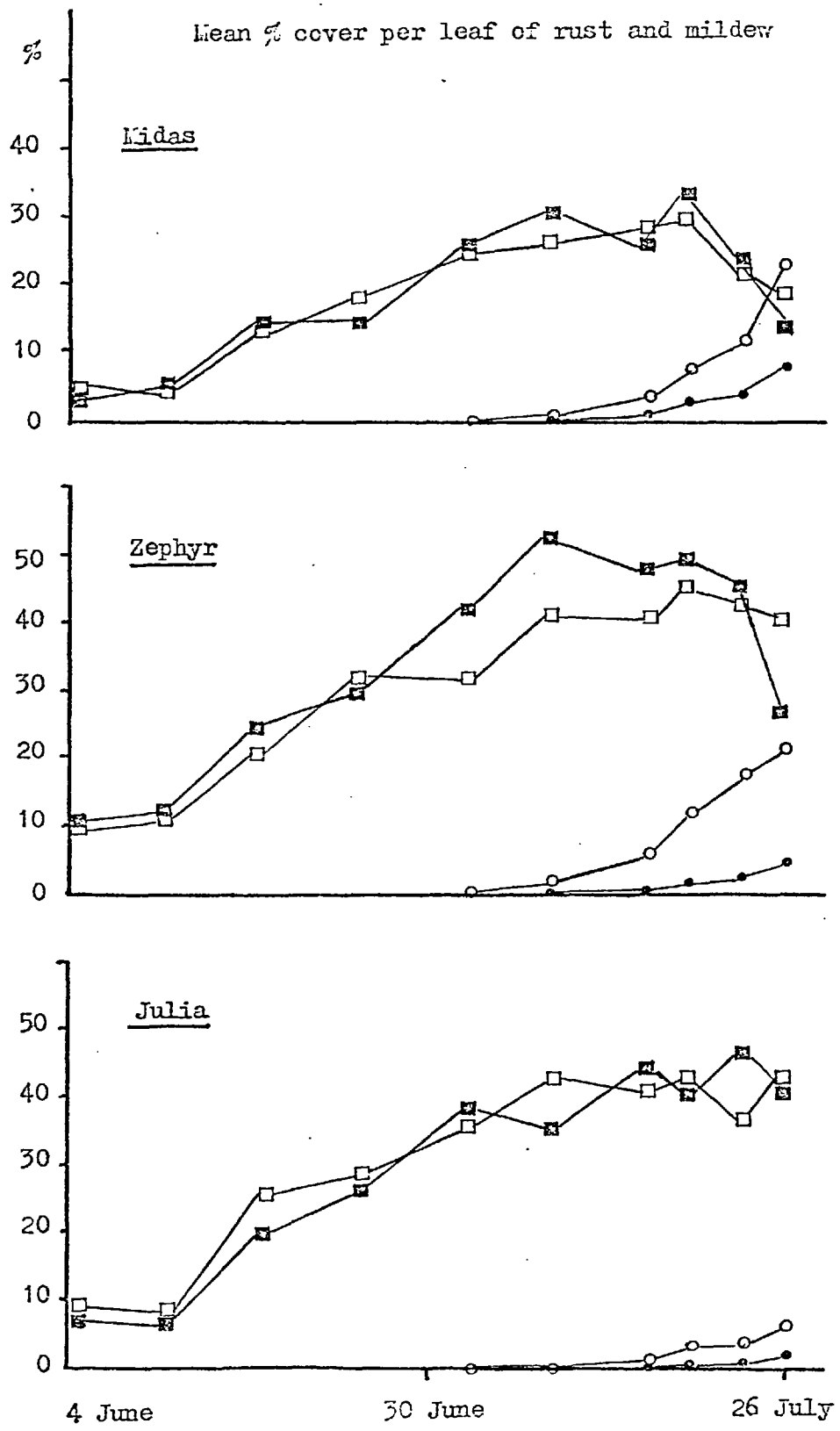
Analyses of variance on the mean percentage cover of mildew at each of the last five assessment dates (Table 43) indicated that mildew developed less on Midas than on either Zephyr or Julia but that the levels to which it developed were not significantly altered by the presence of rust.

When comparable data for mildew development on the flag leaf (leaf 1) and leaf 2 (Tables 44 and 45) were analysed a similar result was obtained. Mildew developed less well on Midas than on either Zephyr or Julia but only on 16 July on the flag leaf was there any significant effect on mildew of the presence of rust. There were similar trends in the data from leaves 3 and 4 but little rust developed on these leaves until they began to senesce so these data have not been analysed.

Because mildew levels did not differ significantly on a cultivar with or without rust it might be expected that the rates of

Figure 10 Disease assessments 1974

Mean % cover per leaf of rust and mildew



Mildew { □ Treatment 1 (mildew only)
■ Treatment 3 (mildew and rust)

Rust { o Treatment 2 (rust only)
• Treatment 3 (rust and mildew)

Table 43 Development of mildew, July 1974.

(see also Appendix Table 61)

Mean percentage cover per leaf at each assessment†

Date: July		9	16	19	23	26
Midas	A	26.06	27.98	30.20	21.63	18.42
	B	30.23	26.88	33.77	23.60	13.67
Zephyr	A	42.44	41.14	45.55	42.33	40.89
	B	52.74	48.26	49.54	40.48	26.77
Julia	A	42.13	41.01	42.63	35.98	42.65
	B	35.29	43.33	40.25	46.59	40.35
SE ±		4.04	4.29	3.51	5.79	6.93

A = development of mildew only (treatment 1)

B = development of mildew in the presence of rust (treatment 3)

Analysis of variance.	'F' values July 1974				
Source of variation	9	16	19	23	26
Presence of rust	0.593	0.640	0.360	0.58	1.55
Cultivar	11.59**	9.48**	9.98**	6.99**	7.09**

Values significant at $P \leq 0.01$ indicated by **

† Values are the sums of the percentage cover on each leaf(6 plants in each of 3 replicate plots) divided by the number of leaves.

Table 44 Development of mildew on the flag leaf (1), 1974.

(see also Appendix Table 62)

Mean percentage mildew per leaf for each assessment date.

Date:		9 July	16 July	19 July	23 July	26 July
Midas	A	0.28	0.81	0.97	1.31	1.31
	B	0.72	4.45	5.03	3.11	2.24
Zephyr	A	4.80	9.97	14.08	21.73	22.93
	B	4.04	10.56	14.03	13.17	13.13
Julia	A	3.95	7.47	13.23	12.67	10.08
	B	5.89	16.31	21.61	28.68	21.94
S.E. ±		1.35	1.90	2.45	4.80	4.97

A = Development of mildew only (treatment 1)

B = Development of mildew in the presence of rust(treatment 3)

Analysis of Variance	'F' values				
Source of variation	Date: 9	16	19	23	26 July
Presence of rust.	0.24	7.88*	4.26	0.54	0.05
Cultivar	6.41*	13.56**	18.95**	8.29**	6.14*

Values significant at $P \leq 0.05$, and 0.01 denoted by * and** respectively.

Table 45 Development of mildew on leaf 2

(see also Appendix Table 63)

Mean percentage per leaf for each assessment date.

Date:		9 July	16 July	19 July	23 July	26 July
Midas	A	8.58	11.53	14.45	14.83	16.11
	B	11.07	15.99	21.56	19.00	17.50
Zephyr	A	35.53	46.38	53.51	62.92	58.80
	B	46.49	55.79	56.26	56.97	45.42
Julia	A	36.70	40.28	54.31	59.49	75.21
	B	34.89	45.96	52.08	65.25	58.75
S.E. ±		4.27	6.27	4.92	5.25	11.22

A = Development of mildew only (treatment 1)

B = Development of mildew in the presence of rust (treatment 3)

Analysis of Variance 'F' values

Source of variation	Date: 9	16	19	23	26 July
Presence of rust	1.24	1.63	0.39	0.10	1.07
Cultivar	30.53**	19.66**	35.91**	45.52**	10.54**

Values significant at $P \leq 0.01$.

Fig 11 Mildew development on the Flag leaf

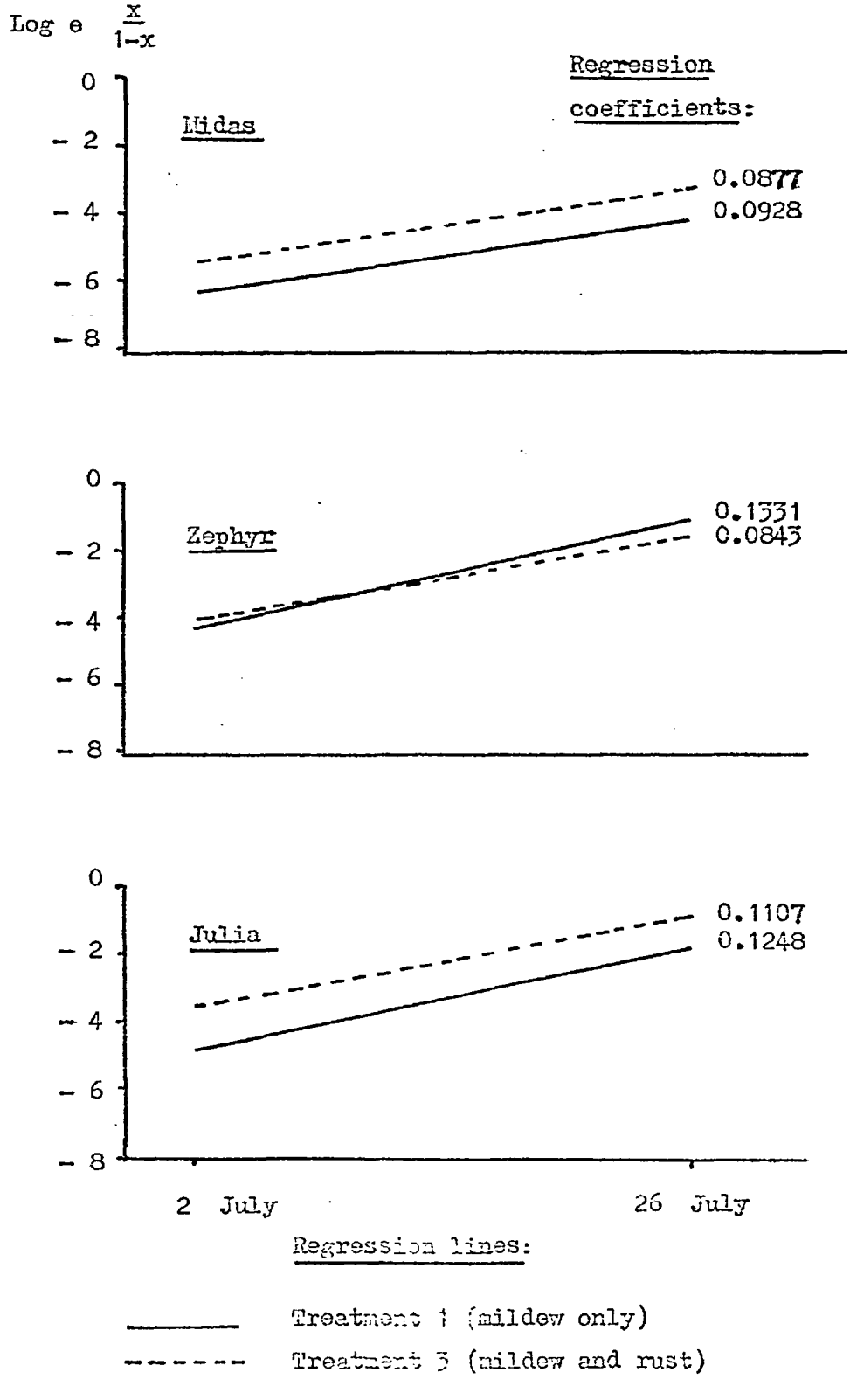
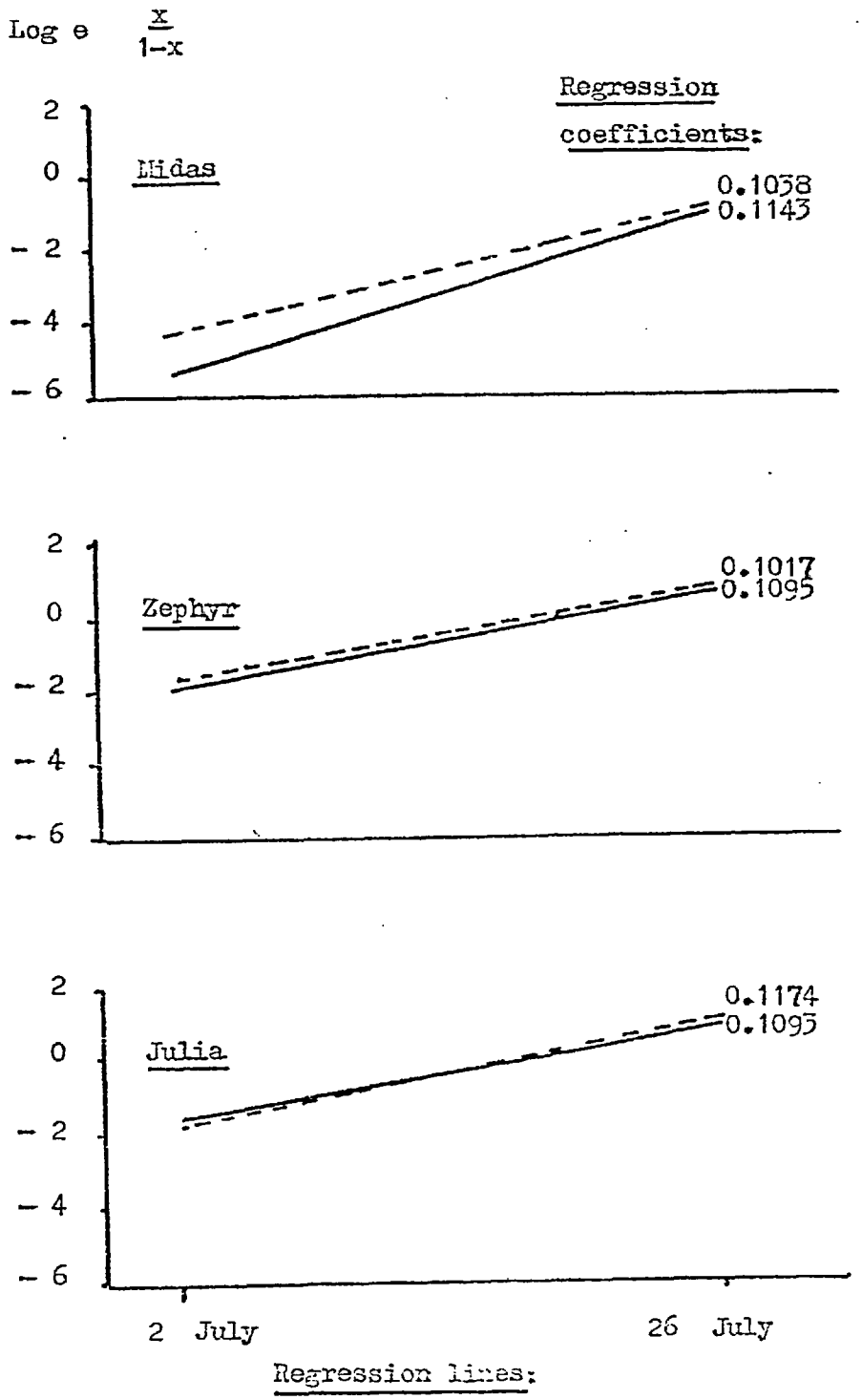


Fig 12 Mildew development on leaf 2 (Flag leaf = 1)



mildew development would also be similar in the two situations. This proved to be so. There were no significant differences in infection rates of mildew with or without rust on the flag leaf (Fig 11) and on leaf 2 (Fig 12) of the three cultivars as determined by the regression of $\log_e \frac{x}{1-x}$ against time (Van der Plank, 1963).

b. Development of rust

Figure 10 indicates that at most assessments more rust was recorded on plants where mildew was controlled (Treatment 2) than on plants where it was not controlled(Treatment 3), and that there was more rust on Midas and Zephyr than on Julia. Analyses of the data for assessments throughout July,(Table 46) showed that these differences were statistically significant.

Mildew also affected rust early in the season when the amounts of rust were so small that numbers of pustules per leaf were recorded. Data for the period, 25 June to 9 July, (Table 47) show that at each assessment there were more rust pustules on plants where mildew was controlled than on plants where mildew was not controlled. Only on 25 June did the cultivars have a significant effect on the rust levels, less pustules being found on Julia than on either Zephyr or Midas. Similar cultivar differences were apparant on 2 and 9 July but due to the large amount of variation they were not significant statistically. The presence of mildew thus appeared to have a greater effect on the rust than the resistance of the cultivars. Cultivar differences were apparent from an analysis of the bulked data for the three assessments(Appendix Table 66).

The amounts of rust on the flag leaf and leaf 2 of plants where mildew was controlled were higher than on similar leaves of mildewed plants (Tables 48 and 49). These differences were larger on Zephyr

Table 46 Development of rust, 1974.

(see also Appendix Table 66)

Mean percentage per leaf.

Date:		9 July	16 July	19 July	23 July	26 July
Midas	A	0.67	3.42	7.95	11.35	22.96
	B	0.09	0.83	2.64	3.61	7.80
Zephyr	A	1.78	6.00	11.96	17.89	21.59
	B	0.10	0.97	1.98	2.52	4.63
Julia	A	0.53	1.42	3.82	4.04	6.66
	B	0.00	0.30	0.92	0.89	1.96
S.E. ±		0.32	0.56	1.57	2.53	3.97

A = Development of rust only (treatment 2)

B = Development of rust in the presence of mildew (treatment 3)

Analysis of Variance.

	'F' values.				
Source of variation	Date: 9 July	16 July	19 July	23 July	26 July
Presence of mildew	11.84**	40.13**	22.3**	17.97**	14.32**
Cultivar	2.79	10.81**	4.39*	4.81*	4.32*

Values significant at $P \leq 0.05$ and 0.01 indicated by * and ** respectively.

Table 47 Early development of rust. 1974.

(see also Appendix Table 67)

Mean number of rust pustules per plot for each assessment date.

Date:		25 June	2 July	9 July
Midas	A	1.67	11.00	184.3
	B	3.67.	77.40	890.0
Zephyr	A	1.00	7.70	158.7
	B	14.30	126.70	2393.3
Julia	A	0.00	3.67	29.4
	B	0.00	26.37	403.2
S.E. ±		2.40	23.41	413.2

A = Treatment 3 (rust and mildew)

B = Treatment 2 (rust only, mildew controlled)

Analysis of Variance	'F' values		
Source of variation	Date: 25 June	2 July	9 July
Presence of mildew	6.78*	13.18**	10.72**
Cultivar	5.24*	2.49	3.46

Values significant at $P \leq 0.05$ and 0.01 indicated by * and ** respectively.

Table 48 Development of rust on flag leaves, 1974.

(see also Appendix Table 68)

Mean percentage cover per leaf for each assessment date

Date:		16 July	19 July	23 July	26 July
Midas	A	1.19	4.75	7.58	13.88
	B	0.86	2.81	4.78	6.90
Zephyr	A	1.72	5.88	10.06	14.28
	B	1.08	2.72	2.96	3.83
July	A	0.16	1.28	1.27	6.05
	B	0.47	1.06	1.10	2.58
S.E. ±		0.35	1.24	1.66	2.92

A = Development of rust only (treatment 2)

B = Development of rust in the presence of mildew (treatment 3)

Analysis of Variance 'F' values.

Source of variation	Date: 16	19	23	26 July.
Presence of mildew	0.59	3.07	6.17*	8.54*
Cultivar	4.88*	3.72	6.48*	2.40

Values significant at $P \leq 0.05$.

Table 49 Development of rust on leaf 2, 1974.

(see also Appendix Table 69)

Mean percentage rust per leaf for each assessment date

Date:		9 July	16 July	19 July	23 July	26 July
Midas	A	0.25	2.50	8.22	11.69	24.20
	B	0.17	1.11	3.72	5.00	7.88
Zephyr	A	1.67	4.25	13.78	25.22	26.04
	B	0.50	1.11	2.45	2.50	5.42
Julia	A	0.17	1.48	3.75	3.81	10.35
	B	0.00	0.31	1.03	0.69	4.00
S.E. ±		0.29	0.71	2.04	3.32	4.88

A = Development of rust only (treatment 2)

B = Development of rust in the presence of mildew (treatment 3)

Analysis of Variance	'F' values				
	July, 1974				
Source of variation	Date: 9	16	19	23	26
Presence of mildew	2.47	10.59**	13.81**	15.97**	14.79**
Cultivar	2.63	3.12	4.03*	6.14*	2.83

Values significant at $P \leq 0.05$ and 0.01 indicated by * and ** respectively

and Midas than on Julia due possibly to the resistance of Julia to rust. Similarly the size of the differences appeared to be larger on Zephyr than on Midas which is possibly due to the greater resistance of Midas to mildew than Zephyr. The amounts of rust on these two leaves of Julia was lower than on Zephyr or Midas of the same treatments for two assessment dates on each leaf. The same effects of cultivars and mildew on the amount of rust on leaf 3 and 4 were observed (Appendix Tables 70 and 71), but the rust was obviously lower in amount than on leaves 1 and 2.

The size and density of the rust pustules on mildewed leaves (leaf 2) was also significantly less than on similar non-mildewed leaves. Rust pustules were also smaller and less dense on Julia than on Midas or Zephyr (Table 50).

The rates of rust development per mean leaf (Fig 13), as indicated by regressions of $\log_e \frac{x}{1-x}$ against time, appeared to be reduced in the presence of mildew on Zephyr and Julia (where mildew was high) but these differences proved not to be statistically significant. Similar effects were also seen on the flag leaves (Fig 14) and leaf 2 (Fig 15) but were also not sufficiently large to be statistically significant. On Midas, possibly because of its relatively high resistance to mildew and relatively high susceptibility to rust, the infection rates did not appear to be reduced by the presence of mildew as much as on Zephyr or Julia.

Table 50 Development of rust on leaf 2

(see also Appendix Table 73)

		Mean no. rust pustules per cm^2	Mean area of pustules (mm^2)
Midas	A	97.4	0.037
	B	184.9	0.045
Zephyr	A	121.7	0.034
	B	206.3	0.045
Julia	A	58.4	0.030
	B	72.9	0.038
S.E. ±		23.0	0.0017

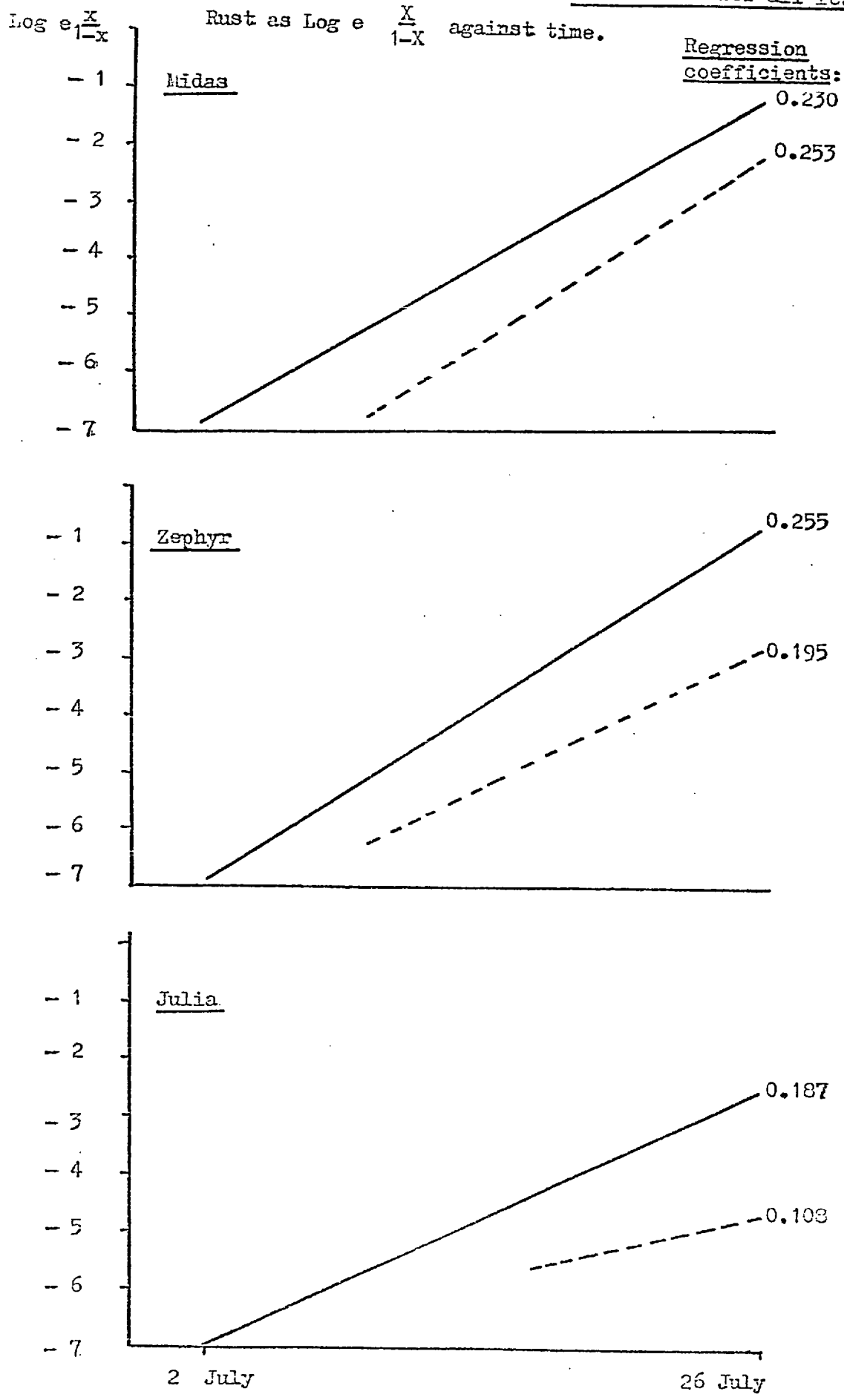
A = Treatment 3 (rust and mildew)

B = Treatment 2 (rust only, mildew controlled)

Analysis of Variance	'F' values.	
Source of variation	Rust pustule no.	Rust pustule size.
Presence of mildew	14.32**	36.93***
Cultivar	7.31**	8.26**

Values significant at $P \leq 0.01$ and 0.001 denoted by ** and *** respectively

Fig 13 Development of rust, July 1974. Mean values for all leaves.



——— Treatment 2 Rust only; - - - - - Treatment 3 Rust and mildew.

Fig 14 Development of rust on the Flag leaf.

Rust as Log e $\frac{x}{1-x}$ against time.

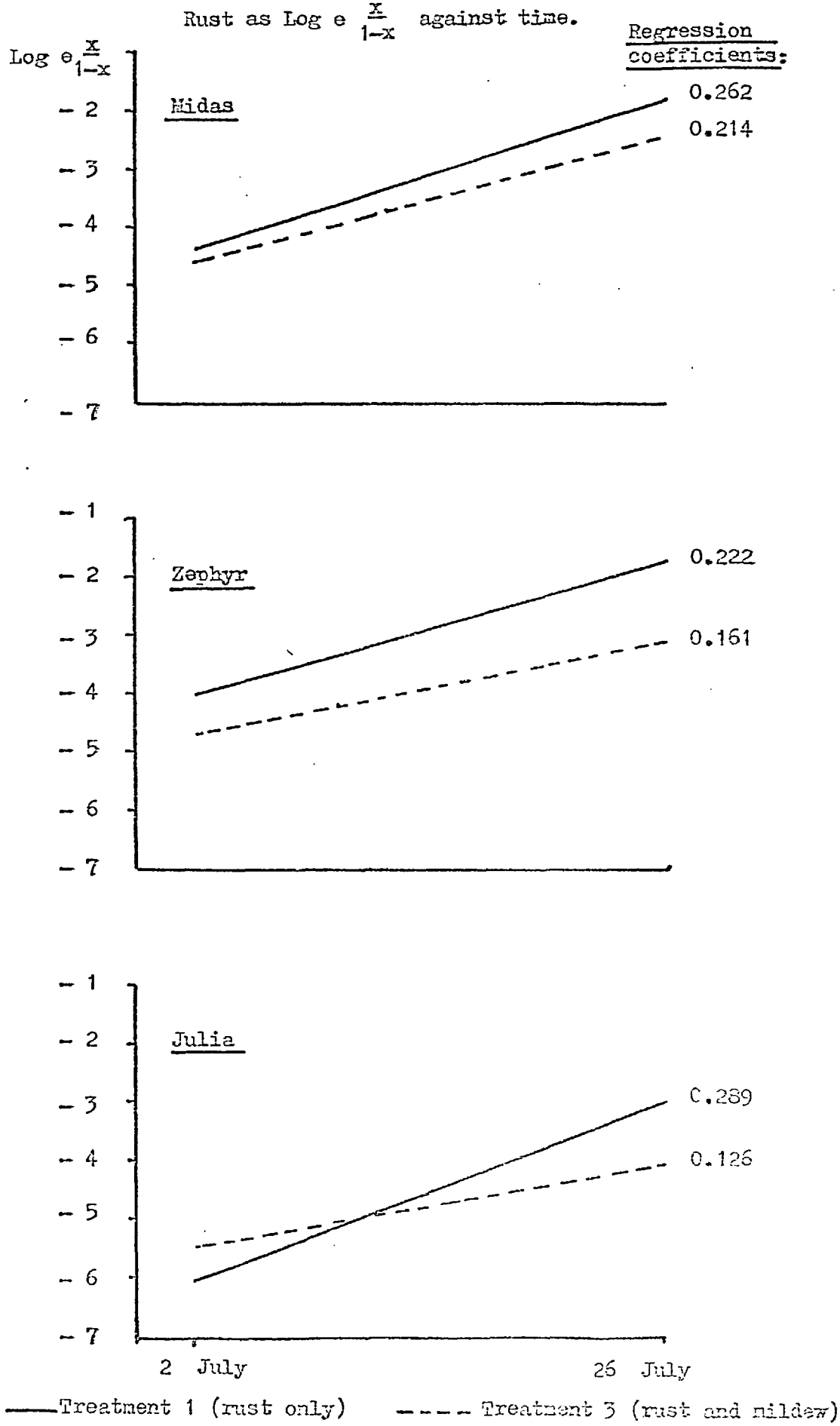
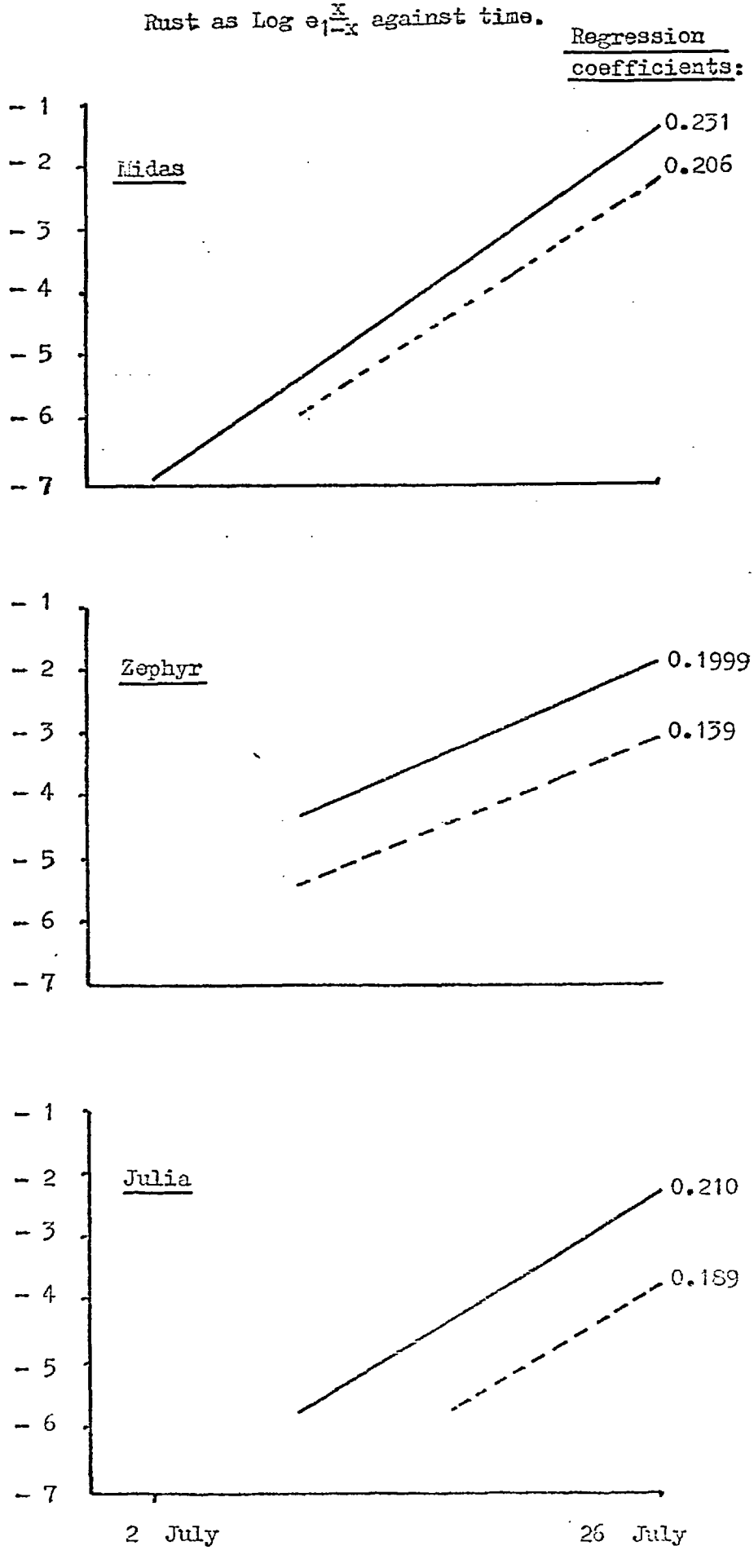


Fig 15 Development of rust on leaf 2

Rust as Log $e_{\frac{1}{1-x}}^{x}$ against time.



c. Disease development on late tillers.

On 6 August, 10 days after the last disease assessment on the main tillers, the level of rust and mildew were assessed on the flag leaves of late tillers. This was considered to give an approximate representation of the disease situation as it was at the end of the 'main tiller' season (Table 51)

There was a significant effect on the mildew levels of treating plants with Calirus earlier in the season. On plots where rust was controlled (Treatment 1) the mildew developed to higher levels on the late tillers than in plots where rust was not controlled (Treatment 3). This effect was most pronounced on Midas possibly due to this cultivar's resistance to mildew and susceptibility to rust.

Also the levels of rust on the late tillers were significantly higher on plants treated with ethirimol earlier in the season. This indicates that the interactions between rust and mildew on the main tillers were maintained on the late tillers after the leaves on the main tillers had senesced.

3. Yield

Blocks, cultivars and fungicide treatments all significantly affected yield. A further analysis of the treatment effects indicated that both mildew and rust reduced yield but, overall, there was no significant mildew x rust interaction. Effects on individual

Table 51 Disease development on late tillers.

(see also Appendix Table 73)

Mean percentage cover of rust and mildew on flag leaves.

Treatment:	1. Mildew only	2. Rust only	3. Mildew and Rust mildew	rust
Midas	8.17	33.0	0.06	25.3
Zephyr	4.68	14.0	2.37	8.53
Julia	6.30	15.3	2.93	6.70

Analysis of effects

	'F' values (variance ratio)
On mildew	
Presence of rust	17.93^{**}
Cultivar	0.61 n.s.
On rust	
Presence of mildew	15.03^{**}
Cultivar	40.94^{***}

Significant effects at $P \leq 0.01$ and 0.001 denoted by ** and *** respectively

cultivars were determined by comparing ranked means in a Duncan's Multiple Range test. With all cultivars, yields from plots in which rust and mildew were allowed to develop were significantly lower than from plots with neither disease. With Zephyr and Julia mildew only (treatment 1) significantly reduced yield while rust only did not. With Midas rust reduced yield while mildew did not; effects obviously related to the differing susceptibilities and resistances to rust and mildew of the three cultivars (Table 52).

The lack of significant losses due to rust on Zephyr were probably due to there being patches of the barley crop which suffered from magnesium defficiency and this gave rise to non-random variation.

The two diseases also affected the size and composition of the grain as the results of 1000 grain weight determinations showed (Table 53)

Analysis of variance on the 1000 grain weights for each cultivar showed that mildew was the main cause of the reduction in grain weight on all cultivars. On Zephyr rust also significantly reduced the grain weight and with Midas and Julia there were significant rust x mildew interactions. This indicated that rust and mildew when in combination (treatment 3) had less effect on the grain weight than when they developed singly(treatments 1 and 2).

The results of sieve tests (Appendix Table 76) showed similar but not significant effects of rust and mildew on the grain size.

Table 52 (see also Appendix Table 74) — Field trial 1974 — Silwood Park.

Mean plot yields (Kg.)

Treatment	Cultivar: Midas	Zephyr	Julia	
1. Mildew only	2.86	1.64	2.15	
2. Rust only	2.65	2.31	2.46	S.E. ± 0.086
3. Mildew and rust	2.71	1.74	2.15	
4. Neither disease	3.27	2.45	2.71	

S.E. ± 0.074

Table 53 (see also Appendix Table 75) — Field trial 1974 — Silwood Park.

Mean 1,000 grain weight (g.)

Treatment	Cultivar: Midas	Zephyr	Julia	
1. Mildew only	29.52	33.38	33.02	
2. Rust only	31.09	34.79	35.83	
3. Mildew and rust	30.62	32.01	35.03	S.E. ± 0.424
4. Neither disease	36.00	38.70	40.52	

S.E. ± 0.367

B. Field Trial 1975 Silwood Park.

1. Introduction and Materials and Methods.

A second field trial was carried out in 1975 identical to that in 1974 in respect of cultivars, treatments, design and methods of disease assessment(P137). It differed from the 1974 trial as follows:

a. Spore trapping was performed at the same intervals as the disease assessments, the method used (Jenkyn, 1973; Onursal, 1975) involved placing vertically above each plot a metal rod (0.6cm diam.), which was protected from the rain, and on which was attached a band of cellophane (20mm wide) coated with a mixture of vaseline and 12% parafin wax in toluene. These rods were replaced weekly and the cellophane removed, mounted on a slide using clear nail varnish and examined microscopically. The numbers of uredospores of P.hordei and conidia of E.graminis were counted in five traverses 25mm long (circumference of rod) and 0.24mm wide (field diameter).

b. Weed control was practiced when the barley was at the six-leaf stage by spraying with Dichloroprop with M.C.P.A.(cornox, Boots Ltd.). This controlled the broad-leaved weeds. The monocotyledenous weeds were rogued by hand from within the plots and treated with Paraquat (ICI Ltd.) on the paths between plots.

c. Since the aim of the field trial was to investigate the interactions between rust and mildew neither disease was assessed until both were present in the field.

Inter-plot Midas strips were sown on 25 March and plots on 21 April. The Midas strips and plots within trestments 2 and 4 were sprayed with Milgo (350g ethirimol/hectare) On 19 June; plots within treatments 1 and 4 were sprayed with Calirus (2.781Kg 75% w.p./hectare) on 4 July.

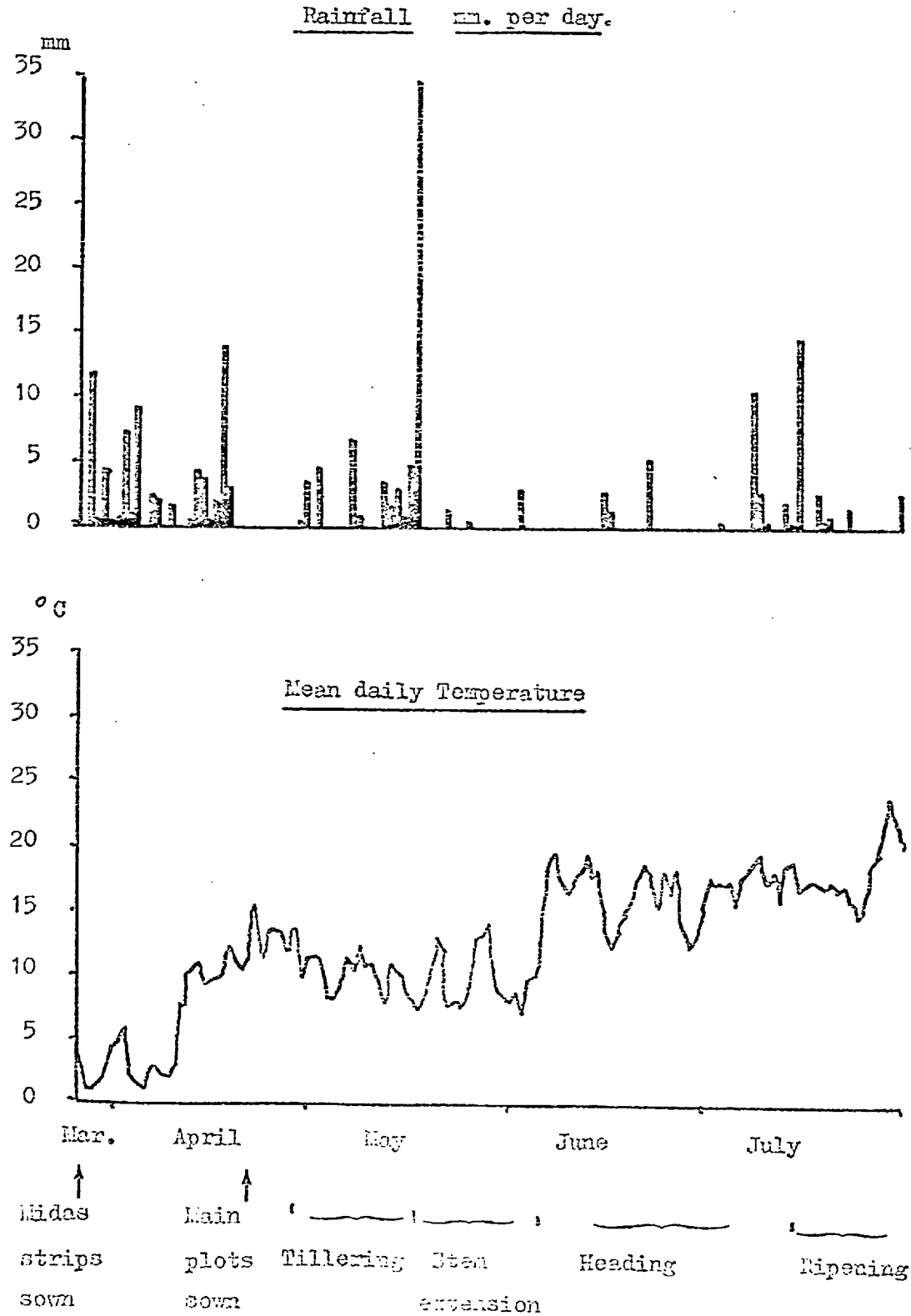
2. Crop development and Weather.

The meteorological data for the 1975 growing season are summarized in Fig 17 and were obtained from Silwood Main Meteorological site, latitude 51° 28' N.

Following the sowing of the interplot Midas the weather was very cold and wet; snow was observed on several occasions, this resulted in only moderate and delayed emergence. The cold wet period continued into the the middle of April making conditions unsuitable for the later sowing of the main plots until the third week of April. During the remainder of April and first half of May there were frequent rains and moderate temperature which gave rise to good emergence and crop growth within the plots. However from then until the begining of July($1\frac{1}{2}$ months) there were only 23mm of rainfall and very high temperatures were recorded. During this period the barley suffered from drought and began heading prematurely. Rainfall was intermittent during the remainder of July and the temperatures remained high until the barley was harvested.

Fig 16 Meteorological Data Silwood Park 1975.

Latitude 51° 28' N



Growth stages of main plots.

3. Mildew development.

Analysis of variance of the mean percentage cover of mildew per leaf (Appendix Table 77) indicated that mildew developed to the same levels on rusted leaves as on non-rusted leaves of all three cultivars and at each assessment. In general the mildew developed less well on Midas than on Zephyr or Julia, an effect significant on 7 and 14 July. At this stage in the season the mildew epidemic was in decline and the last leaves were begining to senesce. This gave rise to variable results and as a consequence no determinations were made of the rates of mildew development. The levels at each assessment however were not different on rusted and un-rusted plants and it is thus unlikely that the rates of development differed with these treatments.

4. Rust development.

As in the previous season the initial progress of rust was assessed by counting the number of pustules per leaf, and then by assessing the percentage cover as the level of disease increased. Analysis of variance of the results(Table 54) indicated that the presence of mildew reduced the amounts of rust recorded at each assessment. (ie. rust developed less well on mildewed than non-mildewed leaves.) This effect was more apparant on Midas and Zephyr than on Julia where the level of rust was lower.

Since rust did not reach sufficiently high levels to be recorded as percentage cover until 21 July no determinations were made of the rate of development of rust.

Table 54 Field Trial 1975 Silwood Park.

(see also Appendix Table 78)

Rust per leaf †

Treatments:	Date July	Mildew only	Rust only	Mildew and rust	Neither disease
Midas	7	0.70	1.33	0.33	0.50
	14	0.41	1.29	0.19	0.61
	21	0.44	2.15	1.29	0.33
Zephyr	7	0.00	1.37	0.19	0.25
	14	0.15	1.02	0.23	0.14
	21	0.06	3.82	0.73	0.15
Julia	7	0.17	0.30	0.13	0.38
	14	0.07	0.04	0.02	0.22
	21	0.05	0.88	0.04	0.50

† Mean pustule number per leaf on 7 and 14 July; mean percentage cover per leaf on 21 July.

Analysis of variance	'F' values (variance ratios)		
Source of variation	Date: July 7	14	21
Presence of mildew	8.271*	11.067**	5.636*
Cultivar	2.112	5.379*	2.365
S.E.±	0.339	0.230	0.773

Values significant at $P \leq 0.05$ and 0.01 denoted by * and ** respectively

5. Spore trapping

Analysis of variance of the numbers of uredospores trapped over the three weeks 7 to 28 July (Table 55) indicated that the use of fungicides (ethirimol and Calirus) significantly altered the numbers of uredospores trapped. Further analysis showed that in plots where rust developed in the absence of mildew (Treatment 2) there were ~~significantly~~ appeared to be more uredospores trapped than over other plots either where rust was controlled by Calirus or where it developed in the presence of mildew(Treatment 3). There was a significant mildew x rust interaction indicating that the presence of mildew significantly reduced the number of uredospores trapped.

Due to the large amount of variation in these results no significant cultivar effect was detected. This may also reflect an effect of the Midas inter-plot strips 'blanketing' the area with uredospores. In general however it appeared that greater numbers of uredospores were trapped over plots of Midas and Zephyr than over Julia.

The numbers of conidia of E.graminis trapped were also variable. The use of ethirimol to control mildew and the presence of rust appeared to decrease the numbers of conidia trapped but these observations were not statistically significant. In contrast to the uredospore counts the numbers of conidia trapped were affected by the cultivar used. More were trapped above Midas than above either Zephyr or Julia. This unexpected result probably reflects the open growth habit of Midas and its later senenscence.

Analysis of variance of the data obtained from each separate assessment for both conidia and uredospores resulted in only one factor

Table 55 (see also Appendix Table 79) Total number of uredospores and conidia trapped per plot 7 to 28 July.

Uredospores.

Treatments:	Mildew only	Rust only	Mildew and rust	Neither disease
Midas	50	105	125	69
Zephyr	81	137	48	36
Julia	43	105	21	40

Conidia

Midas	505	419	443	419
Zephyr	505	333	461	256
Julia	346	240	257	208

Analysis of variance	Variance ratios (F)	
Source of variation	Uredospores	Conidia
Treatments	4.681*	2.416 n.s.
Cultivar	2.190 n.s.	5.976*
Blocks	1.562 n.s.	4.875*

Values significant at $P \leq 0.05$ denoted by *.

causing a significant effect (Appendix Table 80). This was the effect of the cultivar used on the number of conidia trapped from 14 to 21 July. There were fewer conidia trapped over plots of Julia than Zephyr or Midas, probably due to the rapid and early senescence of this cultivar.

6. Yield.

The results of yield determinations (Table 56) indicated that in this season the presence of disease severely reduced yield. Analysis of variance showed that this was due to the presence of mildew. The presence of rust did not significantly reduce yield and there was no mildew x rust interaction. The analysis also showed a significant effect of the cultivar used; as in 1974 Midas yielded more grain than either Zephyr or Julia.

The quality of the grain was also adversely affected by mildew. Rust had no significant effect and again there was no mildew x rust interaction.

These results showed that in this season although rust developed to a high level eventually, it was too late to cause significant effects on yield. Mildew however had the usual effect of reducing both the 1,000 grain weight and the overall weight of grain produced.

Table 56 (see also Appendix Table 81) — Field Trials 1975 Silwood Park.

Mean plot yields (Kg)

Treatments:	Mildew only	Rust only	Mildew and rust	Neither disease
Midas	3.157	3.777	2.487	3.957
Zephyr	1.857	2.500	1.503	2.603 S.E ± 0.163
Julia	2.627	2.770	2.420	2.913

S.E. ± 0.188

Analysis of variance

Source of variation	DF.	Sum of squares	Mean square	Variance ratio (F)
Treatments	3	5.615	1.872	5.913**
Cultivars	2	8.834	4.417	13.955**
Treat x cult.	6	1.030	0.172	0.542n.s.
Blocks	2	0.588	0.294	0.929n.s.
Error	22		0.317	
Total	35	23.030		

Separation of treatment variation

Mildew	1	4.847	4.847	15.379**
Rust	1	0.632	0.632	1.997 n.s.
Mildew x rust	1	0.135	0.135	0.427 n.s.
Error	22		0.317	
Total (Treatment)	3	5.615		

Variance ratios significant at $P \leq 0.01$ denoted by **

Table 57 1,000 grain weights(g), 1975 Field trial.
(see also Appendix Table 82)

Treatments:	Mildew only	Rust only	Mildew and rust	Neither disease	
Midas	34.90	35.43	34.43	35.43	
Zephyr	34.97	35.33	34.17	35.63	S.E. ± 0.212
Julia	36.13	38.07	35.97	37.40	
		S.E. ± 0.245			

Analysis of variance

Source of variation	DF.	Sum of squares	Mean square	Variance ratio(F)
Treatment	3	12.429	4.143	7.704 **
Cultivar	2	27.507	13.754	25.575 **
Blocks	2	1.104	0.552	
Treat x cult.	6	2.486	0.414	
Treat x blocks	6	1.076	0.179	
Cult x blocks	4	0.518	0.129	
Treat x cult x blocks	12	10.209	0.851	
Error	22		0.538	
Total	35	55.329		

Separation of Treatment variation.

Mildew	1	10.890	10.890	20.25 ***
Rust	1	0.320	0.320	0.60 n.s.
Mildew x rust	1	1.210	1.210	2.25 n.s.
Error	22		0.538	
Total (Treatments)	3	12.429		

Values significant at $P \leq 0.01$ and 0.001 denited by ** and *** respectively

Discussion

The experiments described clearly indicate that interactions between P.hordei and E.graminis on barley do exist in the sense that these pathogens develop differently when in combination on a leaf. In greenhouse experiments these interactions were observed on the first and second seedling leaves of Zephyr barley and also on leaves of several other cultivars, so they would appear to be of general occurence. However, the effects seem to be fairly localized and to operate only in the region of pustules on one leaf. In these respects they are similar to those reported by Manners & Gandy (1954) for E.graminis and P.triticina on wheat.

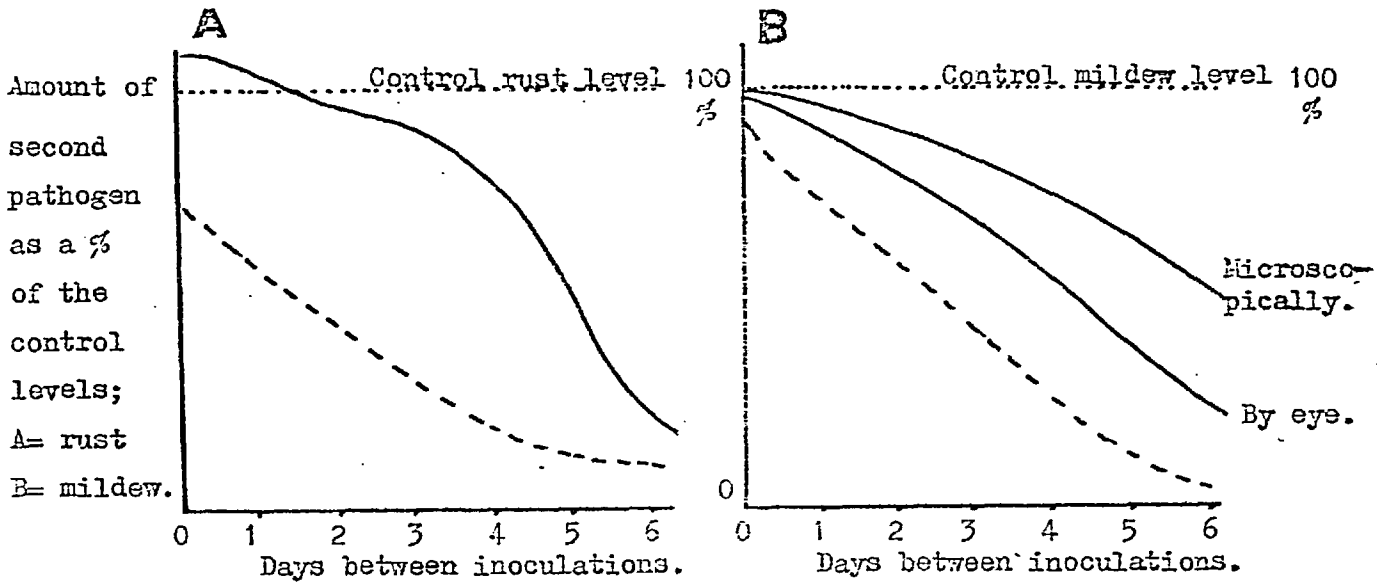
The extent to which E.graminis and P.hordei interact on a leaf depends principally on the cultivar, the amounts of inocula and the time between inoculations. Most data have been obtained with the cultivar Zephyr, which is susceptible to both pathogens, and these are considered first. The general patterns of the interactions which were observed are summarized by Figures A, B and C, these of necessity are oversimplified but they serve to illustrate the salient features. Figures A and B consider the effects of the period between inoculations in the two situations examined. When leaves were inoculated with E.graminis and then within 24h.also inoclated with P.hordei more rust developed than on the corresponding controls (Fig A). With periods longer than 1 day between these inoculations the number of rust pustules was reduced and this effect was most marked with intervals longer than 3 days. Pustule size was affected somewhat differently. The sizes of rust pustules which developed on plants pre-inoculated with

Dual inoculations - Zephyr.

Effect of the length of time between the inoculations:

1- E.graminis 2- P.hordei

1- P.hordei 2- E.graminis

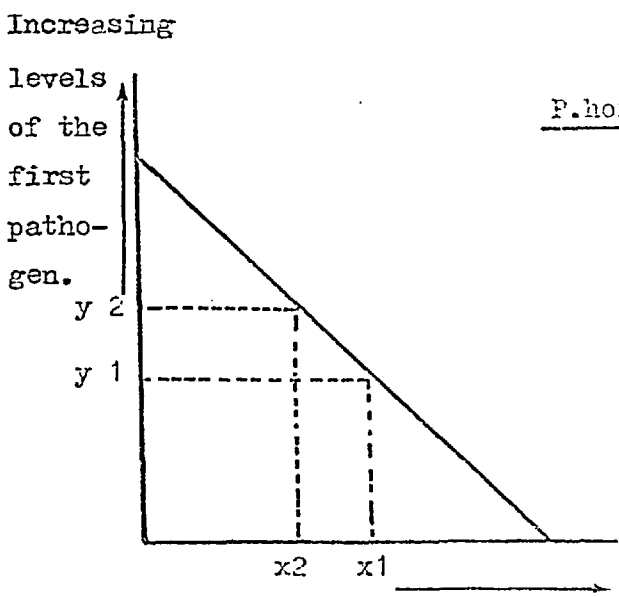


——————— Rust pustule/mildew colony number.

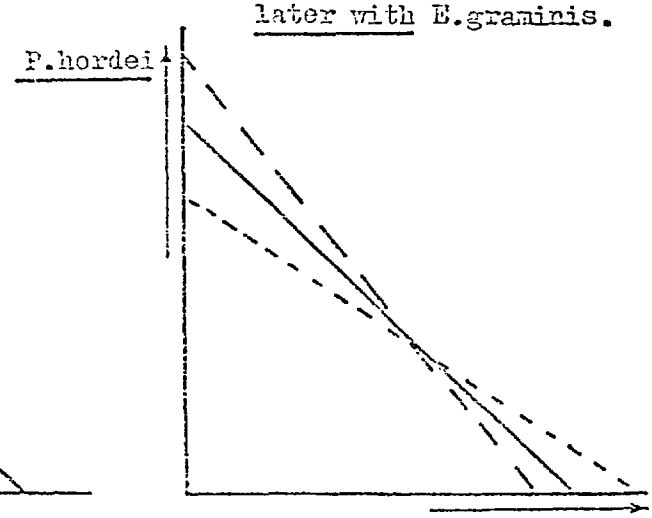
- - - - - - - - Rust pustule/mildew colony size.

Effect of different inoculum levels of the 'first' pathogen:

C Zephyr



D Other cultivars inoculated first with P.hordei and then 4 days later with E.graminis.



Midas — — —

Zephyr ———————

Julia - - - - - - - -

E.graminis were smaller than on the controls. Presumably this was due mainly to competition with mildew but additionally, with intervals between inoculations of 1 day or less, this may also have been due to the greater number of rust pustules.

Pre-inoculating leaves with P.hordei (Fig B) also reduced the number of mildew colonies to an extent determined largely by the time interval between the inoculations. The reductions in mildew were similarly most marked with intervals longer than three days. However, assessments of colonies by the naked eye overestimated the effects on numbers. When leaves were cleared and colonies observed microscopically it was apparent that colony size was more affected than number and limitation of the development of some colonies meant that they were not visible to the naked eye and thus were not recorded, in those assessments.

Fig C gives a much-simplified picture of the effects of different amounts of inocula, assuming for this purpose a standard period between inoculations of 4 days. In brief, increasing the amounts of the first pathogen decreased the development of the second. In extreme cases development of the second pathogen could be completely inhibited. Thus, in one experiment development of rust was prevented by inoculating leaves with very many conidia of E.graminis, though the interval between inoculations was here also increased to 6 days.

Interactions between P.hordei and E.graminis on other cultivars are basically similar to those for Zephyr, differing only in degree. The most interesting changes are those which result from the different susceptibilities of these cultivars to the two pathogens.

The cultivar Midas, for example, is more susceptible to brown rust than Zephyr but less susceptible to mildew. In greenhouse experiments pre-inoculating leaves with P.hordei affected the development of mildew, relative to the control, more on this cultivar than on Zephyr. In contrast, the cultivar Julia is less susceptible to brown rust than Zephyr but of similar susceptibility to mildew. In greenhouse experiments, pre-inoculating this cultivar with E.graminis had, relatively, a greater effect on rust development than did comparable inoculations on Zephyr. These two situations can be illustrated by changing the slope in Fig C to give the modifications appropriate to the cultivars shown in Fig D, and this indicates the complexity of the interaction phenomena.

A special case arises when one cultivar is completely resistant to one of the pathogens and so does not support its growth. Mazurka, for example, is resistant to race B 72/27 of E.graminis and inoculations result only in flecking of the leaf. It might be supposed, therefore, that a subsequent inoculation with P.hordei would result in rust development comparable to that on a control plant untreated with E.graminis. Experiments showed that this was not so. Pre-inoculating leaves of Mazurka with this race of E.graminis reduced the subsequent development of rust to a degree comparable with that observed on Zephyr. Much less effect was seen in a similar type of experiment using Zephyr and a race of E.graminis(B71/98) to which it was not susceptible. Here, there was no visible response of the host to inoculation with E.graminis and very dense inocula were required to produce any effect on rust development.

Further experiments with Zephyr in which leaves were pre-inoculated with Diplocarpon rosae or Uromyces phaseoli, neither of which produced a visible response on inoculated leaves, could not demonstrate any affect on either mildew or rust development from subsequent inoculations with E.graminis or P.hordei.

Clearly a range of effects of one pathogen on a second which alights on a leaf can be demonstrated. What is of particular interest is how these effects are brought about. Some of the different effects obtained with first inoculations of E.graminis or P.hordei appear to result from the different growth habits of these two fungi on the leaf. Conidia of E.graminis germinate readily and the fungus quickly establishes a first haustorium in the epidermis of the leaf and the grows over the leaf surface. In contrast, the uredospores of P.hordei do not germinate so readily and the process of successful establishment of an infection involves not only growth of the germ tube to and into a stoma but the development of a substomatal vesicle and primary infection hyphae. This establishment is thus a much more prolonged affair than that of E.graminis, but once the fungus is established it can grow in the leaf away from any direct physical competition from other organisms on the leaf surface. This vunerability of P.hordei in its early stages of development on the leaf might account for the lower numbers of rust pustules in situations where leaves were first inoculated with E.graminis, although direct counts of germ-tubes reaching stomata and forming sub-stomatal vesicles were not possible because of the mildew growth.

The smaller size of the rust pustules which formed cannot, however, be accounted for in this way. Probably these result from

competition for nutrients within the leaf for, although the two fungi develop at different sites, they must draw on the same nutrient supply. There are indications however that the distances over which these nutrient effects operate are fairly small. Thus, when the upper surface of Zephyr leaves were inoculated with E.graminis and after 4 days the lower surface was inoculated with P.hordei, there was no significant reduction in rust pustule size. Pustule number was also not affected giving some support to the idea that mildew growth has some physical effect on the establishment of P.hordei. In contrast to this, the available evidence indicates that the initial establishment of E.graminis was not affected by the pre-inoculation with P.hordei. Instead, the main effect was a slowing down of colony growth, in many instances to the point where the colony was so small that it was not visible to the naked eye(and so not recorded by visual assessments) and the production of conidia was considerably reduced. This suggests that the nutrient supply to the developing mildew colony was impaired in some way. This is not altogether suprising for the epidermis in which the mildew haustoria develop is devoid of chloroplasts and is therefore, itself dependent on the palisade and mesophyll and it is within these tissues that the rust develops and from which it derives its nutrients.

The assumption here is that in these respects the interactions observed reflect a competition for nutrients within the leaf. If this is so then it ought to be possible to alter the extent of the interactions by changing the level of nutrients in the leaf. That this could be done by floating leaf segments on sucrose or by treating plants with maleic hydrazide gives some support to this hypothesis.

At first sight, it would appear difficult to invisage such a hypothesis to account for the effects on rust which were observed when the cultivar Mazurka was pre-inoculated with E.graminis (race B72/27). However, even in this instance there were indications that the nutrient status of the leaf was affected in the area of the flecks which the inoculation with E.graminis produced, and it is worth noting that in other experiments with 'non-compatible' fungi where no visible host reaction was induced there was a marked lack of effects. Some further evidence can be derived from the experiment with Zephyr, in which the growth of the first pathogen(E.graminis or P.hordei) was arrested by a specific fungicide(ethirimol or Calirus respectively) before the leaf was inoculated with the second pathogen. In these instances, the extent of the interaction between the two fungi was much less than in situations where the first pathogen was allowed to continue its development. These results also suggest that on Zephyr, growth of the first pathogen does not induce the formation of compounds inhibitory to the second pathogen, or at least not the formation of materials that can remain in the leaf for appreciable periods. However, it is possible that such compounds are formed in Mazurka inoculated with E.graminis(race B72/27) and that these contribute to the reduction in the subsequent growth of P.hordei.

Whether changes in nutrient levels of the leaf also result in the increased number of rust pustules when inoculations of E.graminis and P.hordei follow each other closely is a matter of speculation. There are quite dramatic changes in the water-soluble-carbohydrate levels following inoculations with E.graminis and these could make the tissue temporarily more suitable for the growth of

P.hordei. The relatively short period over which these high levels of carbohydrates are maintained could account for the somewhat variable occurrence of this effect on rust.

Although it is possible to demonstrate various types of interaction between E.graminis and P.hordei in greenhouse experiments depending on the cultivars used and types and amounts of inocula with which they are treated, the main points of practical interest are whether such interactions occur in field-grown barley and if so, what effects these have on the development of these pathogens in crops and on yields. Normally in the field the climatic conditions limit the range of interactions which occur. Whereas in Spring barley mildew develops substantially at tillering, brown rust appears later usually at heading, so that generally infection by E.graminis preceeds that by P.hordei. Data from the two trials clearly showed that on mildewed plants rust develops later and to a lesser extent than on plants kept relatively free of mildew with ethirimol. Effects on the number and the size of rust pustules were observed. Similar data from late-developing tillers substantiated the main results but, additionally showed that in the presence of rust the amount of mildew was reduced. This effect was not seen on the main tillers and in this respect the results differ from those reported by Simkin & Wheeler (1974) for the trial in 1971.

In the present trials the extent to which mildew affected rust was partly determined by the susceptibilities of the cultivars to the two pathogens as was indicated in the greenhouse experiments and discussed above. Confirmatory evidence for the reduction in rust on mildewed plants was obtained from the vertical cylinder spore traps. Catches of rust uredospores over plots in which rust only was allowed

to develop (treatment 2) appeared to be significantly higher than all other treatments. This implies that the presence of mildew (in treatment 3) reduced the rust to a level comparable to that in plots treated with Calirus (treatment 1 and 4). It seems likely that if plots had been isolated and not adjacent to continuous sources of rust uredospores ie. the Midas strips, that the effects of mildew on rust and so on uredospore numbers might well have been more marked. However, the benefit of the Midas strips in ensuring that brown rust developed substantially within the trial outweighed such considerations.

In both trials mildew caused significant yield losses but brown rust affected yield significantly only in 1974. The extent of the losses were much greater with mildew than with brown rust and this reflected the relative severities of the two diseases. In the analysis of the yield data there was no significant mildew x rust interaction but the yields of the cultivars with both diseases or with one disease show interesting trends.

In 1974, the effects of rust and mildew on the yield of all cultivars appeared to be less when they developed together (treatment 3, Midas 17.1; Zephyr 28.98; Julia 20.7, % yield reductions) than when they developed separately, (treatments 1 mildew only: Midas 12.5; Zephyr 33.03; Julia 20.7, and treatment 2 rust only: Midas 18.96; Zephyr 5.71; Julia 9.23, % yield reductions). In 1975, the effects of rust and mildew on Julia appeared to be additive(mildew 9.8; rust 4.9; rust with mildew 16.9 % reduction) and on Zephyr and Midas yield reductions where both diseases occurred together were greater than might have been expected (Zephyr: mildew 28.7; rust 4.0; mildew with rust 42.3; Midas: mildew 20.2; rust 4.5; mildew with rust 37.1, % reductions).

However, the lack of significant mildew x rust interaction demands that further conclusions of this type be made with caution. The overall results of the field trials emphasize the complexity involved in these disease interactions, especially where one disease (ie brown rust) develops late in the growth of the crop.

It may well be that other disease combinations would be more amenable to this type of study, for example, E.graminis and P.striiformis on wheat. The most interesting situations are obviously those in which one or other of the two pathogens are controlled with a specific fungicide since this always poses the question will this allow the second pathogen to develop to a greater extent. It is this type of question which farmers ask and it is one which plant pathologists find difficult to answer because so often they have been concerned only with the study of one disease despite the fact that one disease on its own is a very rare occurrence.

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Appendix Tables

APPENDIX 1

Inoculation with rust uredospores and mildew conidia.

(a) The distribution of uredospores in the apparatus shown in Fig. 1, was examined by taking five leaves at random from a freshly inoculated batch of plants. Sellotape impressions were taken from the leaves, these were stained in lactophenol cotton blue and examined microspically. Uredospores were counted in five sets of five fields.

An analysis of variance showed that there was no significant difference between spore loads on any of the leaves (Table 1).

It was found that 15 - 30 mg of fresh uredospores diluted to 1g with talc as inoculum in the apparatus gave a fairly even infection of between 75 and 150 pustules per leaf.

(b) A series of inoculations with E.graminis were carried out to determine the most useful inoculum level and also to check the reproducibility of the method. Ten pots of plants were inoculated with mildew conidia derived from five infected leaves each with 70% of their area mildewed. The conidia were allowed to settle for 20 minutes and then two pots of plants were removed (Treatment A, Table 2). The remaining eight pots were re-inoculated with conidia from a further set of five mildewed leaves and two pots were then removed after 20 minutes (Treatment B) and so on, to give three more treatments (C,D and E). The number of pustules were then counted on all plants after 7 days. The results (Table 2) suggested that little was to be gained by using more than fifteen leaves so for most inoculations five to ten mildewed leaves were used, (each with $\leq$ 70% mildew).

Table 1 Distribution of Uredospores

Leaf no.	Total number of uredospores in 5 microscopic fields					Totals
1.	8	3	5	3	3	22
2.	2	7	2	8	0	19
3.	6	5	6	4	5	26
4.	11	4	5	2	3	25
5.	4	7	5	0	3	19
Totals	31	26	23	17	14	

Analysis of variance

Source	DF	Sum of Squares	Mean Squsre	F-test
A	4	8.560	2.14	0.28997 N.S.
B	4	37.360	9.34	1.265 N.S.
A X B	16	110.240	6.89	
Error			7.38	
Total		156.160		

A = rows, B = columns.

Table 2 Test inoculations: conidia of E. graminis

Number of infector leaves	Number of mildew colonies per leaf	Mean
5	46,45,31,30,88,55,56,45,48,46,78,56.	52
10	122,65,83,113,76,91,65,67,104,85,94.	87
15	182,114,141,96,97,78,107,105,119,104,195.	114
20	115,126,128,98,125,177,110,122,187,115.	131
25	159,286,245,120,164,138,163,205,145,212.	184

Infector leaves were 60-70 % covered with 10-day old mildew infection

Table 3 Effects on rust development of pre-inoculating leaves with E. graminis.

Time between inoculations	Number of pustules/colonies per leaf	Mean
Inoculated with *E. graminis* only (control)	53,118,476,136,192,199,163,279, 155,159,299,134,39,189,55,63, 86,185,45,83,236,140,103.	158
2h between inoculations		
Mildew	28,44,50,133,61,251.	95
Rust	554,529,555,285,307,275.	418
Control rust only	414,278,272,348,261,318.	330
1 day between inoculations		
Mildew	272,218,285,356,260,196,103,222,164,128,252.	196
Rust	31,195,146,251,121,133,157,137,187,91,312.	161
Control rust only	170,342,399,127,149,204,403,287,373,224,154, 332.	264
2 day between inoculations		
Mildew	122,184,372,78,168,298,220.	182
Rust	245,234,247,283,203,249,319.	254
Control rust only	253,163,297,225,227,209,115.	213
3 days between inoculations		
Mildew	61,182,44,124,53,269,141.	125
Rust	558,568,511,421,378,426,399.	466
Control rust only	320,488,409,236,420,553,372.	400
4 days between inoculations		
Mildew	266,87,356,139,124,116,24,27.	160
Rust	12,4,4,6,17,7,36,12.	12
Control rust only	84,134,339,212,264,81.	191

Table 4 Effects of dual inoculations with E.graminis and P.hordei on first seedling leaves of Zephyr barley: inoculation 1, P.hordei; inoculation 2, E.graminis.

Treatment		No. colonies/pustules per leaf	Mean
Control: Mildew only		100,100,100,100,100,100,100, 90,100,100,100,100,100,100.	99.5
Dual inoculation at day 0	Mildew	95,95,100,100,35,85,95,100,70, 60,90,95,90,95,60.	84.3
	Rust	150,200,150,200,190,250,170,260, 300,200,300,300,300,400,500.	261.3
Control: Rust only		110,120,150,190,150,170,80,250, 190,170,120,160,140100.	150.0
Control: Mildew only		16,67,51,74,67,80,51,56,65,88, 75,43,66,74.	62.5
Dual inoculation at day 2	Mildew	26,32,21,22,54,25,60,19,20,23, 32,15,30.	29.2
	Rust	100,100,110,170,13,170,80,100,110, 150,80,120,100.	97.7
Control: Rust only		160,130,150,180,130,250,130,180, 180,100,100,110,125,150.	148.3
Control: Mildew only		98,47,48,56,42,48,47,41,40,43, 51,54,57,27.	49.9
Dual inoculation at day 4	Mildew	15,24,34,2,8,14,6,15,42,17,5,9,30, 16,34.	18.1
	Rust	150,75,100,100,150,170,150,160,100, 150,200,50,120,50.	128.3
Control: Rust only		100,100,100,90,100,120,90,150,100, 60,110,100,150,70.	102.9
Control: Mildew only		142,171,159,137,148,151,123,123, 115,190,144.	145.7
Dual inoculations at day 6	Mildew	45,20,90,40,10,79,30,41,19,38,66,72.	45.8
	Rust	90,60,30,50,12080,110,100,150,110 50,75.	84.6
Control: Rust only		60,60,60,90,50,40,40,30,70,60,60.	56.4

Table 5 Effects of dual inoculations with E.graminis and P.hordei on first seedling leaves of Zeohyr barley: inoculation 1, E.graminis; inoculation 2, P.hordei.

Treatment		No. colonies/pustules per leaf	Mean
Control: Mildew only		100,100,100,90,100,90,100,100, 100,100,100,100.	98.3
Dual inoculations at day 0	Mildew	60,90,100,100,100,100,100,50,100, 100,100,100,100,100,95.	93.0
	Rust	180,175,190,210,190,255,250,300, 350,320,190,250,190,190,300.	236.0
Control: Rust only		115,145,135,140,120,110,200,190, 185,170,140,130.	148.3
Control: Mildew only		100,100,100,100,100,100,100, 100,100,100,100,100,100,	100.0
Dual inoculations at day 2	Mildew	95,100,100,100,100,95,100,100, 100,100,100,100.	99.2
	Rust	85,72,54,32,12,17,29,41,18,19, 21,30.	35.8
Control: Rust only		100,100,150,150,75,100,125,130, 150,170,90,100,150.	122.3
Control: Mildew only		100,100,100,100,100,100,100, 100,100,100,100,100,100,100.	100.0
Dual inoculations at day 4	Mildew	100,100,100,100,100,100,100,100, 100,100,100,100,100,100,100,	100.0
	Rust	0,0,0,10,0,0,0,0,0,0,0,8,0,0,0.	1.2
Control: Rust only		15,67,49,50,7,12,34,59,6,5,19,16.	30.7
Control: Mildew only		100,100,100,100,100,100,100,100, 100,100,100,100,100,100,100,100.	100.0
Dual inoculations at day 6	Mildew	100,100,100,100,100,100,100, 100,100,100,100,100,100,100.	100.0
	Rust	0,0,0,0,0,0,0,0,0,0,0,0,0,0.	00.0
Control: Rust only		10,40,5,30,20,10,70,70,50,60,40,60.	38.7

Table 6 Effects of dual inoculations with E.graminis and P.hordei on the second seedling leaf of Zephyr barley: inoculation 1, P.hordei; inoculation 2, E.graminis.

Treatment		No. colonies/pustules per leaf	Mean
Control: Mildew only		90,90,75,85,80,80,90,70,95,95, 95,85,70,75.	90.7
Dual inoculations at day 0	Mildew	75,50,75,75,85,60,85,70,50,80, 50,70,100,50.	70.7
	Rust	200,200,200,190,250,200,230,300, 300,200,150,300,300,400,500.	261.3
Control: Rust only		115,340,175,210,160,240,130,450,250, 300,210,200,380,200.	240.0
Control: Mildew only		40,87,90,48,68,72,78,60,78,90, 81,52,69,67.	70.0
Dual inoculations at day 2	Mildew	32,30,31,22,51,26,82,35,46,41, 34,35.	40.1
	Rust	200,200,150,180,120,250,180,160, 160,150,110,110,100.	151.1
Control: rust only		400,200,160,170,300,160,190,220, 170,170,150,250,300.	224.3
Control: Mildew only		117,45,62,89,57,77,80,68,51,58, 53,61,72,58.	67.7
Dual inoculations at day 4	Mildew	36,52,41,30,25,29,29,21,26,24, 21,39,10,12,17.	27.5
	Rust	220,100,150,150,140,150,200,120, 160,200,200,170,180,120.	120.5
Control: Rust only		250,190,160,190,200,200,180,190, 190,150,220,150,190,300.	197.1
Control: Mildew only		138,144,172,191,187,182,171,163, 190,116,160.	165.0
Dual inoculations at day 6	Mildew	40,60,50,70,20,39,115,120,71, 94,79,121.	73.3
	Rust	150,50,50,50,20,100,100,160,200, 190,120,100.	107.5
Control: Rust only		110,120,150,170,130,135,140,120, 110,100,120.	127.6

Table 7 Effects of dual inoculations with E.graminis and P.hordei on second seedling leaves of Zephyr barley: inoculation 1, E.graminis; inoculation 2, P.hordei.

Treatment		No. colonies/pustules per leaf	Mean
Control: Mildew only		95,80,80,95,75,90,85,85, 70,75,80,90.	84.2
Dual inoculations at day 0	Mildew	80,80,80,70,70,80,75,90, 75,100,90,90,75,90,90.	82.33
	Rust	270,250,190,300,230,350,170,300, 300,350,250,200,210,220,270.	257.3
Control: Rust only		120,330,190,170,200,230,140,380, 370,210,210,190.	228.3
Control: Mildew only		60,80,95,85,90,85,85,70,90, 95,90,100,90.	78.1
Dual inoculations at day 2	Mildew	65,70,80,95,65,95,70,65, 50,60,70,80.	72.1
	Rust	126,146,55,72,77,61,62,59, 41,17,113.	73.7
Control: Rust only		400,250,400,120,300,300,300,250, 250,150,350,300.	274.0
Control: Mildew only		90,95,95,100,100,95,95,85,90, 90,85,85,80,85.	90.7
Dual inoculations at day 4	Mildew	95,90.85,100,95,95,100,90,90,90, 70,95,75,90,95.	90.3
	Rust	5,6,17,16,4,8,15,9,0, 18,25,20,36,47,4.	15.3
Control: Rust only		88,82,17,92,43,3,40,30,10, 17,8,17,42.	37.6
Control: Mildew only		100,95,95,95,95,95,90,80,50, 50,70,85,85,95.	79.5
Dual inoculations at day 6	Mildew	90,95,100,95,90,90,95,100,95, 95,70,50,80,75.	90.0
	Rust	10,10,30,20,40,40,50,0,1,0,5,0,10, 0,15.	15.4
Control: Rust only		40,70,20,80,100,120,130,200, 200,200,150,160.	122.5

Table 8 Efects of dual inoculations with E.graminis and P.hordei; on different leaves of Zephyr barley

Replicate no.	Leaf no.	No. rust pustules per leaf	
		Seedling leaves(1,2,3) pre-inoculated with E.graminis	Seedling leaves not pre-inoculated
1	4	14,26,24,8,95.	26,8,23,22,25,81.
	5	20,18,25,22,16.	12,21,7,33,2,29.
2	4	14,8,12,7,8,35,29.	22,1,15,60,10.
	5	44,40,59,44,21,10,25, 16.	45,1,73,81,22.
3	4	5,12,3,10.	0,1,21,10,25,62,99.
	5	16,34,18,57.	44,36,51,76,46,122,102.
4	4	1,0,5,2,3.	1,1,0,0,25,0.
	5	16,34,3,13,141.	40,20,50,31,47,25.
5	4	0,0,2,7,0,0,0,10,14.	6,9,0,0,0,25.
	5	0,1,5,8,14,0,5,0,0,8.	28,10,5,10,10,41.

Table 9 Effects on mildew development of pre-inoculating leaves of different cultivars with P.hordei.

Cultivar (and rust level as pustules per leaf)	No. mildew colonies per leaf	
	Leaves pre-inoculated with P.hordei	Leaves not pre-inoculated.
Sultan (2-300)	7,1,14,4,2,2,54,28, 79,0,41,3,9.	83,68,51,84,91, 87,91.
Berac (250-300)	0,1,3,5,0,2,18, 4,5,2,1,7,9.	86,100,21,39,49, 68.
Midas (1-200)	5,3,2,0,5,1,0,4,0,0, 8,1,6,4,1,0,1,3.	14,18,66,35,34, 8,30,16.
Julia (2-300)	0,2,11,27,15,12,16, 5,17,6,8,14.	58,22,36,42, 12,52.
Zephyr (2-300)	11,0,9,6,10,14,15,3,4, 1,31,12,10,0,0.	109,138,68,42, 88,109,75.

Table 10 Data used in Multiple regression analysis

Effects on mildew development of pre-inoculating leaves with P.hordei.

Period between inoculations (days)	Rust level on treated plant, mean no.of pustules per leaf from c 10 plants	Mildew level on treated plant, mean no. of colonies per leaf from c 10 plants	Mildew, % of level on control leaves
0	45	137	78
0	62	47	67
0	25	142	81
0	28	76.8	110
2	22.3	53.8	82
2	32.1	69.9	131
2	27.6	43.7	66
2	14.5	46.3	87
4	40.3	153	105
4	43.2	90.5	93
4	10.6	161	108
4	23.4	95	97
6	30.0	27	59
6	54	14	76
6	38	27	59
6	14	13.5	73
2	42.3	15.2	70
2	34.6	11.2	92
2	12.3	10.6	87
2	63.3	8.8	44
4	30.6	16.2	68
4	18.5	22.1	73
4	18.8	12.7	53
4	18.6	19.7	65
0	58.3	29.1	74
0	8.7	9.1	66
0	11.6	37.4	95
6	126.9	19.8	21
6	10.8	78.6	71
6	26.7	43.9	39
2	33.6	75.3	38
2	147.8	14.2	30
1	33.8	37.9	75
0	207.2	59.2	86
4	375.0	1.4	15
4	275.0	0.1	1
4	250.0	0.2	2
4	175.0	0.5	5
4	150.0	0.5	5
4	175.0	3.7	14
4	125.0	2.6	10
4	37.5	17.1	66
4	50.0	14.5	56
.25	150.0	16.5	64
1	225.0	9.4	36
2	275.0	6.6	25
3	275.0	3.0.	11
4	275.0	72.0	27
0	150.0	84.3	84
2	87.7	29.2	46
4	128.3	18.1	36
6	84.6	45.8	31

Table 11 Data used in Multiple regression analysis
Effects on rust development of pre-inoculating leaves with E.graminis.

Period between inoculations (days)	Mildew level on treated plant, mean no. of colonies per leaf from c 10 plants	Rust level on treated plant, mean no. of pustules per leaf from c 10 plants	Rust, % of level on control leaves
0	151.0	86.6	137
0	131.0	25.0	53
0	52.5	40.5	64
0	56.9	28.7	61
2	34.5	44.4	90
2	31.9	15.0	71
2	29.5	64.9	132
2	30.0	20.2	95
4	36.0	150.0	75
4	40.0	58.0	138
4	24.4	143.0	71
4	24.4	33.0	100
6	92.4	7.0	13
6	90.8	5.8	19
6	48.8	3.2	5
6	54.9	4.4	14
2	46.5	2.6	48
2	43.7	4.7	188
2	44.2	7.3	135
2	49.6	2.7	108
2	39.1	85.9	128
4	200.0	3.2	16
4	200.0	7.0	36
4	200.0	3.7	26
4	200.0	0.5	3
0	36.2	18.0	272
0	35.4	37.1	223
0	12.5	7.8	118
0	18.9	16.2	97
0	47.9	274.9	123
0	94.5	417.5	126
1	196.0	160.1	60
2	182.0	254.2	119
3	124.5	465.9	116
4	159.6	12.3	6
0	186.0	256.0	159
2	198.4	35.8	29
4	200.0	1.2	35
6	200.0	0.0	0

Table 12 Inoculation of leaves with E.graminis followed immediately by inoculation with P.hordei.

	Rust pustules per cm^2 leaf	Mean
Leaves pre-inoculated with E.graminis	56.4,41.4,37.5,46.4,48.0,34.4, 30.8,22.6,30.8,40.2,49.1,60.7,14.3, 67.3,48.4,44.4,49.0,71.4,66.2, 115.4,44.4,35.9,28.6,53.8,42.1, 57.8,32.6,52.0,56.9,35.9,50.7.	47.3
Leaves not pre-inoculated (control)	30.9,15.0,27.7,41.6,30.1,23.3, 14.4,39.5,26.6,26.1,30.1,18.5,39.7, 15.4,30.9,23.4,5.0,8.0,31.5, 14.3,32.1,47.1,29.3,29.1,50.2, 54.8,40.0,44.1,66.9,42.4,24.9.	37.5

Table 13 Dual infections of rust and mildew; Effects in different parts of the leaf of pre-inoculation with E.graminis.

Leaf section	Inoculum:-	Number of colonies/pustules per cm^2 Mildew: E.graminis only	Mildew: E.graminis + Rust: P.hordei	Rust: P.hordei only
Base		1.78,4.0,2.88, 3.21,4.91,1.92, 6.67,3.82,4.6, 4.8,2,5,6.4,3.4, 1.8.	3.7,3.8,3.4, 3.9,2.3,5.8, 6.6,1.4,4, 4.3,2.6,2.4, 3.4,3.9,8.1.	38.2,8.4,13.8, 3.7,0.2,17.8, 21.9,2.5,12,6.4 4.7,14.8,11.6, 8.9.	14.8,17,5.6, 5.6,14.2,2.9, 12.3,16.7,4, 0.9,9.8,16.9, 11.9,21,16.1.
Middle		3,4.4,1.3,1.1, 5,2.8,7.6,4.8, 5.9,3.8,1,5, 4.3,3.4,1.3.	1.9,4.3,3.5,5 2.6,2.2,4.9, 3.4,1,4.2, 2.5,3.1,2.2, 3.8,2.7,2.5.	1.9,23.5,13.8, 21.6,16.1,34.9, 33.6,33.3,26.1, 49.2,37.8,29.7, 34.9,33.7,30.	32,23.8,33.6, 22.5,29.3,19.3, 20,39,22.6,25.6 38.9,26.1,33.8, 37.8,36.9.
Tip		2.8,4.7,4,3, 6.8,4.1,5.4, 6.5,6.1,4.1, 2.3,5.6,4.3, 6.1,4.4.	4.4,3.7,3.5, 2.6,2.0,4.4, 3.9,1.9,3.7, 2.5,3.3,3.6, 2.7,5.5,4.3.	66.7,52.3,47.1, 55.2,39.3,40.7, 75.6,72.2,46, 71.4,64.3,68.8, 37.7,38.7,73.3.	121.2,25,48, 41.5,44.6,37.7, 27.8,35,48.2, 56.9,52.5,65, 33.8,30,55.9.

Table 14 Dual infections of rust and mildew: Effects on rust development of pre-inoculating different leaf surfaces with E.graminis.

Treatments: A P.hordei only; B E.graminis on abaxial surface, P.hordei on adaxial surface; C E.graminis and P.hordei on adaxial surface.

Inoculation with E.graminis day 0, with P.hordei day 4.

Percentage uredospore germination	A	37.6,28.8,33.3,42.9,32.1.
	B	31.6,26.3,27.1,34.8,51.1.
	C	24.1,23.7,22.2,46.0,35.6.
Number of rust pustules per leaf	A	114,116,118,109,84,128,174,77,88,124,144.
	B	98,53,130,183,119,97,136,116.
	C	5,30,90,15,12,56,47,22,18,34,43.
Area of rust pustules (mm^2)	A	.293,.215,.129,.129,.15,.11,.242,.142,.215, .269,.107,.161,.161,.145,.161,.322,.414,.412, .163,.29,.366,.354,.107,.161,.043.
	B	.173,.095,.064,.113,.225,.307..172,.193,.279, .226,.274,.048,.316,.12,.251,.236,.348,.138, .071,.198,.064,.054,.083,.189,.129.
	C	.18,.118,.097,.172,.03,.195,.236,.09,.225, .146,.161,.133,.161,.12,.084,.039,.215,.12, .038,.043,.269,.269,.172,.213,.075.

Table 15 Dual infections of rust and mildew: Effects on rust development of pre-inoculating leaves with large amounts of E.graminis 6 days earlier.

Inoculated with:	Rust development on upper leaf surface		
	% germination of uredospores	No. primary infections †	No. pustules per leaf
P.hordei only	46,47,42,57,61.	6,17,8,22,11.	96,25,36,82,46.
E.graminis and P.hordei	57,53,45,50,52.	0,0,0,1,2.	8,0,1,0,2.

† each value is for 30 microscopic fields.

Table 16 Dual infections of rust and mildew : Effects of pre-inoculating different parts of leaves with P.hordei.

Portion of leaf pre-inoculated with P.hordei	No. mildew colonies per leaf.	Mean
Basal third	32, 22, 65, 44, 95, 18.	59.4
Central third	55, 117, 76, 84, 60, 107.	74.0
Distal third	23, 133, 53, 96, 100, 37, 56.	71.1
Nil(control)	92,88,101,97,75,43,145,100,22,56,63, 57,85,57,70,97,86,118,121,66,108.	83.2

Table 17 Dual infections of rust and mildew: effects on mildew of pre-inoculating leaves with three different inoculum levels of P.hordei.

Number of inoculations with P.hordei.	Number of rust pustules per leaf
1	47,28,36,58,30,35,20,15,5,10,15,6,25,15,30, 20,25,30,35.
2	56,100,140,110,90,120,100,100,90,110,70,80, 60,22,18,80.
3	260,210,240,270,230,280,300,140,170,450,200, 250,250,350,450,420,400.
Nil (control)	

Number of inoculations with P.hordei	Number of mildew colonies per leaf Days from inoculation with E.graminis 10	16
1	59,70,40,67,29,58,66, 105,47,59,95,99,87,52, 100,32,58,74,55.	95,132,57,85,78,110,107, 146,85,105,80,92,52,67, 77,97,86,92,91.
2	52,52,37,31,47,54,28, 44,49,56,64,43,73,57, 140,80.	51,21,67,78,54,54,64, 74,86,68,73,52,55,68, 73,63.
3	51,50,32,53,81,50,62, 40,48,35,46,30,30,16, 15,47,45.	47,46,13,16,50,23,48, 58,80,25,40,14,16,27, 34,15,16.
Nil (control)	61,106,47,53,69,51,66, 70,36,30,50,50,134.	109,90,88,81,98,69,90, 67,64,66,65,120,84.

Table 18 Dual infections of rust and mildew: effect on mildew development of pre-inoculating leaves with a central band of P.hordei.

Zeohyr Distance(cm) from centre of rust.	Area of mildew colonies. (mm^2)	No. of conidiophores(field = $\frac{1}{4}$mm diam) per central field.
Base $2\frac{1}{2}$	1.88,0.67,2.13,2.06,1.37, 1.98,1.33,1.46,2.05,2.66, 2.39,2.05,2.85,2.39,1.46, 1.33.	57,35,68,70,63, 77,75,73,80,76, 68,80,94,74,73, 66.
2	1.85,0.76,1.60,2.22,1.33, 1.46,1.33,1.82,0.67,0.76.	54,17,76,76,75, 73,67,82,45,52.
$1\frac{1}{2}$	0.76,1.67,0.86,1.94,1.98, 1.6,1.33,0.68,1.20.	33,36,35,45,76, 74,76,38,45.
1	0.47,0.31,0.61,0.67,1.33, 0.46,0.34,0.23.	16,12,40,54,63, 33,35,10.
0 (centre)	0.17,0.04,0.61,0.33,0.15, 0.40.	13,3,34,13,13, 14.
1	1.05,1.33,1.33,0.53,1.14, 0.34.	54,38,54,10,51, 46.
$1\frac{1}{2}$	1.33,1.52,1.60,0.95,0.95, 1.05,0.67,1.33.	54,56,22,16,45, 36,10,41.
2	2.66,1.24,0.95,0.86,2.39, 1.37,2.39,0.61,1.37.	72,28,20,30,55, 54,69,14,72.
$2\frac{1}{2}$ Tip	1.33,1.33,0.76,1.32,1.86, 0.76.	56,36,26,69,69, 22.
Control leaves (no rust)	2.28,1.88,1.88,0.76,1.59, 1.05,1.05,1.46,1.14,0.38, 2.13,0.95,1.73,1.52,1.37.	85,89,75,65,85, 64,45,66,68,49, 92,70,84,79,60.
Number of rust pustules in each band.	56,49,75,130,33,38,26.	

Table 19 Dual infections of rust and mildew: effects on mildew development of pre-inoculating leaves with P.hordei.

Leaf impressions 1 day after inoculation with E.graminis. Inoculated with:	Mildew development. % germination of conidia per leaf.(means)†	% appressorium formation	% colony establishment
E.graminis only (control)	16.8,15.8,14.3, 16.7,12.4.	66.6,60.8,64.5, 50.0,85.7.	-
E.graminis and P.hordei.	28.1,26.3,15.4, 17.8,13.7.	75.0,52.4,82.6, 87.0,68.8.	-
Leaf impressions 3 days after inoculation with E.graminis.			
Inoculated with:			
E.graminis only (control)	19.2,17.3,20.6, 17.6,15.8.	81.9,88.8,76.4, 80.0,81.4.	72.1,50.0,38.2, 38.3,71.4.
E.graminis and P.hordei.	22.1,18.4,13.8, 15.6,11.3.	71.4,59.3,53.6, 98.3,65.8.	71.4,46.9,43.3, 84.5,24.0.

Leaves sampled 6 days after inoculation with E.graminis.	Area of mildew colonies (mm^2)	Number of conidiophores per central field(¼mm diam.)
Inoculated with: E.graminis only	1.98,1.41,2.64,1.98,0.85, 0.94,1.89,2.26,1.73,2.20, 3.30,1.89,2.42,2.26,1.57, 3.08,3.85,1.73,4.18,3.08, 2.64,2.07,2.26,2.45,3.52.	43,43,63,57,48, 46,54,41,50,38, 67,63,55,48,36, 53,56,53,58,62, 58,66,56,53,46.
E.graminis and P.hordei.	1.10,1.04,2.26,0.94,2.26, 1.26,1.32,1.13,1.89,1.26, 1.51,1.89,1.89,0.69,1.26, 2.64,1.63,1.98,2.42,1.89, 1.73,4.02,0.76,0.56,0.38.	29,39,28,6,21, 30,18,29,20,23, 36,21,36,9,19, 27,25,49,23,31, 23,36,26,30,16.

† Values are means from 150-200 conidia per leaf

Table 19 (continued)

Leaves sampled 8 days from inoculation with *E.graminis* used to inoculate healthy leaves.	Number of mildew colonies per leaf	Total area of leaves(cm^2)
Inoculum from leaves infected with:		
E.graminis only	56,21,27,26,27,1,16, 28,4,10,15,20,23,57.	58.76
E.graminis and *P.hordei*.	11,20,16,8,5,13,9, 21,12,14,12,8,12,5.	50.57.

Table 20 *Dual infections of rust and mildew: effects on rust and mildew of successive periods of competition.*

Group number	Number of rust pustules per leaf.	Number of mildew colonies per leaf.
3	125 - 150 (estimated)	83,74,20,82,130,105, 140,51,55,203,120,80.
8	55,4,60,32,34,38,21,6, 21,109,48,89,30,15,14.	30,17,19,20,24,27,28,26, 21,35,24,32,18,27,22.
13	8,12,22,16,9,6,22,40, 50,36,59,41,23,34.	20,10,37,25,33,23,34, 22,25,30,29,36.
	Number of rust pustules per leaf	
1	125 - 150.	
4	133,86,68,121,55,43,25,97,50,100,60,96,41,144.	
6	38,85,6,36,58,19,50,27,11,13,37,4,86,6,19.	
9	22,28,51,16,22,21,75,37,60,29,22.	
11	10,24,28,20,38,26,15,16,25,22,14.	

Table 20 (continued)

Group number	Number of mildew colonies per leaf.
2	157,138,121,146,145,172,10[illegible],128,129,152,164,177.
5	18,34,30,27,17,23,26,27,27,20,38,37,26,27,36.
7	27,18,40,29,31,34,20,26,31,31,35,34,33,30,29.
10	34,16,69,62,45,32,48,44,42,51.
12	54,54,44,39,44,45,22,33,32,52,56,29.

Table 21 Dual infections of rust and mildew: efects on mildew of a subsequent inoculation with P.hordei.

Treatment number	Number of mildew colonies per leaf	Mean
1	94,58,63,65,80,53,49,46,68,113,47,71,61,26, 47,57,41,56,45,56,67,40,46.	58.7
2	77,96,56,120,80,59,42,32,71,74,40,31,44,52, 18,93,68,39,45,27,33,40,27.	54.8
3	60,45,41,36,44,33,59,47,96,102,22,34,60,68, 22,22,71,11,19,41,34,53,44.	45.1
4	64,58,57,49,56,58,32,42,39,20,40,38,25,29, 70,19,21,36,23,38,49,40,37.	40.9
5	20,28,30,32,43,31,13,15,34,15,44,21,60,35, 32,49,26,43,38,49,30,15,41,37,65,33,21.	34.1

Table 22 Dual infections of rust and mildew: effects on P.hordei of pre-inoculating leaves with E.graminis.

(a) Reduction in rust

Number of rust pustules per leaf.

Leaves not pre-inoculated with E.graminis (control)	52, 28, 32, 33, 15, 38, 36, 110, 21, 64.
Leaves pre-inoculated with E.graminis.	17, 4, 34, 14, 8, 18, 12, 4, 20, 17, 12, 11, 8, 7.

(b) Mildew development on leaves not inoculated with P.hordei.

Days from inoculation with E.graminis.	Area of mildew colonies (mm^2) mean per leaf.	Mean
4	0.117,0.076,0.057,0.018,0.055.	0.0645
6	0.508,0.564,0.549,0.493,0.452.	0.513
8	2.290,1.912,2.314,2.020,1.891.	2.086
12	4.340,3.785,3.838,3.393,4.550.	3.981
Number of mildew colonies per leaf	87,76,82,97,102,66,56,114,95,87.	86.2

(c) Width of stomatal apertures(μm)mean per leaf (from 40 stomata/leaf)

	Days from inoculation with E.graminis.			
	4	6	8	12
Leaves not inoculated (control)	0.8537	0.800	0.825	2.825
	0.9125	0.9125	0.825	3.200
	1.4250	0.975	0.800	1.950
	0.9000	1.008	0.900	3.025
	0.9500	1.008	0.825	1.625
Leaves inoculated with E.graminis.	0.9750	0.855	1.425	1.575
	1.0750	0.978	1.475	1.175
	0.8375	1.080	1.545	1.300
	1.2250	1.238	1.600	1.200
	0.8750	1.000	1.138	1.025

Table 23 Dual infections of rust and mildew: effect on rust of pre-inoculating leaves with E.graminis 6 days earlier, and of treating plants with ethirimol.

	Leaves not pre-inoculated (control)	Leaves pre-inoculated with E.graminis	
		Not ethirimol treated	Ethirimol treated
No. rust pustules/ leaf: by eye	100,92,119,134, 118,56,144,156, 76,134,156.	96,18,48,8,70, 21,50,57,21,0, 68,64.	140,57,112,93, 43,148,94,78, 113,73,122.
microscopically	107,187,181,155, 108,164,163,250, 171,225,127.	140,144,82,33, 105,164,49,96, 53,122,102.	98,89,137,97, 126,94,154,170, 148,114,121.
Area of rust pustules (mm^2) mean per leaf.	0.10897†† 0.09040 0.08945 0.07615 0.08549	0.05967† 0.04469 0.06209 0.07145 0.03078	0.14753 0.07680 0.10953 0.11198 0.07615

Each figure is a mean of five (†), and ten (††) pustules.

Table 24 Dual infections of rust and mildew: effects on mildew development of pre-inoculating leaves with P.hordei, and of treating plants with calirus.

Treatments:	Basal ½ of leaves				Distal ½ of leaves			
	Mildew colonies per leaf				Mildew colonies per leaf			
	No.of colonies by: eye	No.of colonies by: micro-scope	Area† (mm^2)	C.D.††	No.of colonies by: eye	No.of colonies by: micro-scope	Area† (mm^2)	C.D.††
Leaves not pre-inoculated(control) calirus sprayed.	25	25	1.09	33.0	18	32	1.47	56.4
	16	17	3.68	35.6	17	25	1.23	50.1
	22	32	2.78	47.4	8	19	2.35	20.0
	20	29	2.35	38.4	22	53	3.36	25.0
	20	28	2.59	18.0	23	41	2.80	52.4
Leaves pre-inoculated with P.hordei not calirus sprayed.	20	51	2.76	31.0	1	22	0.25	7.2
	14	34	2.72	19.4	9	42	0.57	4.6
	8	23	1.07	16.4	0	11	0.12	6.0
	13	33	1.82	18.2	6	32	0.19	4.6
	13	18	1.79	14.6	0	36	0.38	5.8
Leaves pre-inoculated with P.hordei and calirus sprayed.	20	34	3.35	26.0	15	58	0.89	15.2
	29	41	2.15	38.4	13	14	1.44	13.6
	27	28	3.44	44.0	17	55	2.15	28.0
	16	18	2.25	28.0	15	23	1.85	25.0
	20	21	1.31	12.6	10	14	0.85	21.0

† mean of five colonies

†† (conidiophore density) mean no. conidiophores per central field from five colonies.

Table 25 Dual infections of rust and mildew: effects of sucrose.

(a) Leaf segments inoculated with E.graminis.

	Pre-inoculated with P.hordei† and floated on:		Not pre-inoculated floated on:
	water	2% sucrose	water
No. conidiophores per central field (0.045 mm^2)	11,6,14,11,6, 3,9,16,6,9, 7,9,4,2,4, 2,4,9,8,3, 9,9,11,14,16,	14,26,19,11,13, 19,19,18,24,19, 11,15,14,18,5, 20,16,12,12,18, 19,22,18,16,18.	23,22,25,13,15, 35,24,21,22,33, 23,27,25,9,28, 22,22,32,13,19, 23,15,24,26,15.

† 70 to 100 pustules on intact leaves.

(b) P.hordei inoculated onto leaves;

	Leaf segments pre-inoculated with E.graminis then floated on:		Not pre-inoculated then leaf segments floated on:	
	water	2% sucrose	water	2% sucrose
Area of rust pustules (mm^2)	.019,.019,.014, .008,.019,.010, .010,.010,.010, .019.	.059,.099,.152 .063,.077,.024, .097,.046,.030, .029,.278,.006.	.175,.123,.160, .192,.133,.152, .186,.164,.274, .228,.299,.234, .129,.223,.201, .314,.111,.140, .222,.238,.312, .239,.285,.192.	.201,.230,.268, .304,.365,.304, .333,.205,.167, .166,.167,.133, .126,.168,.095, .116,.114,.247, .333,.462,.257, .198,.133,.188.
	insufficient pustules			
Total chlorophyll (mg/l)	5.45,5.59,5.33.	4.32,5.38,5.36.	2.38,2.49,1.88.	1.81,1.02,1.57.

Table 26 Dual infections of rust snd mildew: effects of maleic hydrazide.

(a) Leaves inoculated with E.graminis

	Pre-inoculated with P.hordei		Not pre-
	no further treatment(A)	treated with maleic hydrazide(B)	inoculated (C)
No. mildew colonies per leaf.	23,42,16,37,35,39, 28,31,23,36,67,55.	57,76,41,56,53,88, 93,92,76,88.	66,76,103,109,54,74, 43,114,93,81,91,94.
(b)No. mildew colonies per leaf:			
Zephyr	46,20,48,48,22,22, 10,48,14,36,21,20.	85,111,98,69,95, 81,75,62,59,78,58, 47,41,71,60,116,74, 61.	98,105,55,75,73,68, 103,118,64,58,55, 89,49,102,35.
Julia	58,32,39,48,44,44, 21,26,26,35,39,33, 20,15,37,28.	65,64,63,59,54,68, 73,79,61,66,79,87, 54,63,63.	59,64,96,70,101,64, 66,88,76,68,69,49, 53,49.
Mean no. colonies per field from 5 fields($14.5mm^2$)			
Zephyr	0.4,1.0,2.0,1.4, 2.2.***	3.4,3.6,3.2,4.0, 3.4.	3.2,4.0,3.8,3.2, 3.6.
Julia	3.6,2.4,1.0,2.4, 3.2.	3.2,3.6,3.8,3.2, 3.2.	3.6,3.4,3.2,3.4, 3.4.
Mean area of mildew colonies from five colonies per leaf.			
Zephyr	0.319,0.539, 0.58, 1.736,1.914.	3.124,3.498,1.969, 2.310,3.366.	5.286,5.101,5.456, 6.624,6.644.
Julia	2.354,3.036,1.254, 2.222,2.596.	5.455,4.378,4.180, 6.424,3.608.	4.136,5.894,5.71, 5.632,6.644.

*** Significantly different from (C) at $P \leq 0.001$

Approximate rust levels: Zephyr = 150-250; Julia = 150-200.

Table 27 Infections of rust and mildew: alteration in host components. Disease levels recorded; mean no. rust pustules per leaf and mean % cover of mildew.

	Treatments						
Sample number:	A Healthy	B Rust only	C Mildew only	D Rust 1st	D and mildew 2nd	E Mildew 1st	E and rust 2nd
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	5 - 10	0	0	5 - 10	0
4	0	31.2	34.0	26.2	1	31.5	0
5	0	39.0	53.2	43.0	5	32.5	48.0
6	0	46.5	81.7	77.0	10.2	57.2	107.8
7	0	68.2	92.0	55.0	33.7	66.0	123.3

Table 28 Infections of rust and mildew: fresh and dry weight determinations.

Treatments:	Sample number 1	2	3	4	5	6	7
A	0.046†	0.046	0.058	0.053	0.047	0.047	0.049
(Healthy)	10.6††	11.33	9.19	9.0	8.24	8.72	8.799
	0.429†††	0.404	0.629	0.591	0.575	0.536	0.561
B	0.049	0.046	0.061	0.060	0.042	0.058	0.051
(Rust only)	10.0	10.07	10.01	9.58	8.52	10.32	13.09
	0.486	0.426	0.614	0.629	0.495	0.562	0.391
C	0.053	0.054	0.048	0.058	0.060	0.068	0.065
(Mildew only)	10.86	10.93	10.26	10.60	10.04	12.07	17.24
	0.488	0.493	0.463	0.547	0.598	0.543	0.378
D	0.059	0.047	0.053	0.042	0.053	0.065	0.066
(Rust followed by mildew)	10.21	11.38	9.72	10.0	9.26	12.08	15.34
	0.578	0.414	0.545	0.413	0.574	0.540	0.427
E	0.048	0.045	0.056	0.070	0.063	0.073	0.067
(Mildew followed by rust)	10.95	10.32	9.26	11.02	11.2	16.47	25.03
	0.439	0.437	0.605	0.636	0.562	0.441	0.249

† Mean dry weight (g.)

†† % dry to fresh weight

††† Mean fresh weight (g.)

Table 29 Infections of rust and mildew: total chlorophyll (a + b) mg/g dry weight. (mean of three replicates)

Treatment:	Sample number 1	2	3	4	5	6	7
A	15.15	16.06	18.22	19.03	17.03	12.08	9.29
B	16.01	15.97	15.46	14.60	12.05	7.05	3.51
C	13.11	15.27	15.01	11.38	6.15	2.60	1.59
D	15.53	15.68	15.85	14.18	10.59	4.77	2.29
E	13.77	15.35	17.17	9.42	4.87	2.60	2.64

Table 30 Infections of rust and mildew: water soluble carbohydrate determinations. % of dry weight, mean of three replicates.

Treatment:	Sample number 1	2	3	4	5	6	7
A	10.1	5.47	4.1	6.23	5.3	10.07	8.85
B	5.84*	6.97*	5.8*	6.52	5.43	9.3*	9.44
C	9.62	4.82	5.6*	15.47*	8.34*	8.6*	10.43*
D	5.15*	7.3*	7.3*	6.11	8.13*	9.58	6.19*
E	10.63	10.0*	5.5*	14.55*	5.17	6.72*	6.02*
L.S.D. P 0.05	0.75	1.27	1.40	1.40	0.22	0.70	1.36

A = healthy; B = rust only; C = mildew only; D = rust first then mildew; E = mildew first then rust.

* Significantly different from the control mean at $P \leqslant 0.05$.

Table 31 Infections of rust and mildew: effects on total protein content.

Mean % protein of dry weight

Treatment:	Sample number 1	2	3	4	5	6	7
A	11.73	15.00	7.75	7.68	13.07	6.84	5.15
B	12.37	26.9*	35.61*	14.31*	13.15	10.97	13.3*
C	18.05	32.64*	34.19*	37.07*	35.11*	15.42*	26.10*
D	20.43	43.18*	33.33*	33.87*	38.30*	23.23*	15.33*
E	30.56*	36.84*	50.62*	29.35*	24.86*	22.27*	29.57*
L.S.D.	9.56	7.38	14.41	5.11	6.34	5.87	4.62

* Significantly different from the control mean at $P \leqslant 0.05$.

Table 32 Infections of rust and mildew: effects on nucleic acid content. mg/g. dry weight, mean of three replicates.

Treatments:	Sample number 1	2	3	4	5	6	7
A	27.55	24.18	18.73	19.09	17.95	15.26	26.90
B	34.87	24.73	19.16	16.28	16.14	17.13	36.88
C	29.59	24.65	20.99	20.54	16.42	22.54	25.21
D	32.30	25.70	19.44	16.86	14.69	17.19	12.29
E	33.42	25.56	25.25	18.90	16.42	21.52	17.44

A = healthy; B = rust only; C = mildew only; D = rust first then mildew; E = mildew first then rust.

Table 33 Infections of rust and mildew: alteration in host components; water soluble carbohydrates.

Tissues used for extraction:	Water soluble carbohydrate as % of: fresh weight	dry weight
Healthy leaves	0.2232	2.3129
	0.1458	1.5108
	0.2394	2.4808
Rusted leaves	0.2304	2.4240
	0.1640	1.7260
	0.2700	2.8400
Mildewed leaves	0.3262	3.3380
	0.3838	3.9270
	0.3312	3.3890
Isolated rust pustules and surrounding tissue, (1-2mm radius)	0.9920	5.3600
	1.7220	9.3200
	1.2640	6.8400
Remaining parts of rusted leaves (excluding areas 1cm^2)	0.9920	5.1800
	0.9040	4.7200
	0.6240	3.2600
Isolated mildew colonies and surrounding tissue, (1-2 mm radius)	0.8440	3.8400
	0.6800	3.1000
	0.6600	3.0100
Remaining parts of the mildewed leaves (excluding areas larger than 1cm^2 and senescent areas)	0.4500	2.6060
	0.4700	2.7220
	0.6800	3.9380

Table 34 Infections of rust and mildew: alterations in the hosts' components; water soluble carbohydrates.

Sugars identified† (using standard solutions)	Area of spots(mm^2) and r.f. values on chromatographed extracts of:					
	Healthy leaves		Mildewed leaves		Rusted leaves	
	r.f.	r.f.	area	r.f.	area	r.f.	area
Maltose	.176	-	-	.16	55.0	-	-
Galactose	.240	.27	45.0	.26	62.5	.26	42.5
Sucrose	.33	.38	60.0	.29	60.0	.32	65.0
Fructose	.31	.39	55.0	.37	115.0	.38	122.5
Glucose	.42	.44	72.5	.39	105.0	.39	120.0
Ribose	.54	.56	42.5	.53	52.5	.53	42.5
		.66	25.0	.62	27.5	.63	25.0
		.71	12.5	.68	15.0	.68	12.5
		.75	12.5	.71	15.0	.71	17.5
		.82	7.5	.76	10.0	.76	17.5
		.83	12.5	.79	17.5	.78	15.0
		.96	65.0	.94	40.0	.93	60.0
		.99	22.5	.98	35.0	.96	25.0

† Identification by colour reactions with aniline-diphenylamine reagent and r.f. values (Stahl, pages 810-812)

Table 35 The effect of glucose on mildew development on detached leaves.

Concentration of glucose used %	Number of mildew colonies per leaf segment.	
0 (control)	99,152,138,121,100,85,68,122,85,55, 87,141,136,142,88,76,84,89,75,87.	
2	38,86,58,90,62,100,85,92,105,117, 86,97,84,59,107,87,124,68,94,123.	
4	67,88,80,91,36,58,48,66,71,44, 30,58,40,68,45,44,70,28,20,31.	*
6	39,18,58,28,96,56,64,70,66,29, 75,68,56,68,5,8,65,50,32,51.	*
8	18,70,6,0,11,22,76,0,31,58, 20,31,1,66,38,16,46,20,16,18.	*
10	4,0,0,0,0,1,0,0,0,0, 0,1,0,0,0,0,0,0,0,1.	*

* Significsntly different from control at $P \leqslant 0.001$

Table 36 Infection of leaves with E.graminis; effects on hosts' components; antifungal compounds.

Spore germination tests on extracts from healthy and mildewed Zephyr barley.

P.hordei; uredospore germination.

Control distilled water	Healthy extract Dilutions; 1	2	3	4
49.6	0.8	6.0	16.8	33.45
23.0	0.0	3.4	6.38	27.70
40.8	2.4	7.09	8.10	17.65
13.2	0.9	2.80	15.04	25.14
46.2	0.9	4.50	15.0	32.10
	Diseased extract Dilutions; 1	2	3	4
12.4	0.0	2.6	5.04	22.3
17.01	0.0	0.96	5.88	22.4
24.8	1.64	1.48	3.59	13.97
26.5	0.94	8.66	5.50	19.08
	0.0	2.46	2.86	14.40

E.graminis; conidial germination.

Control	Healthy extract: Dilutions; 1	2	3	4
27.36	23.69	20.79	24.03	27.37
25.63	21.88	25.35	34.03	25.30
29.03	23.77	26.85	37.86	37.32
25.85	26.44	32.75	38.46	42.86
28.76	28.74	30.60	28.36	22.66
	Diseased extract: Dilutions; 1	2	3	4
36.67	16.67	38.21	26.95	28.31
29.65	26.29	22.70	20.49	27.94
32.93	23.75	27.27	28.76	26.85
34.13	17.80	30.54	29.68	17.61
26.88	11.18	25.31	21.82	30.65

Values are the percentage that germinated from at least 100 spores.

Table 37 Dual infections of rust and mildew on four cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis or P.hordei respectively.

Mean no. mildew colonies per leaf.

Days between inoculations:		0	1	2	4	6
	Cultivar					
Leaves pre-inoculated with P.hordei	Midas	125,87, 95,84,78, 132,141.	89,56, 113,66, 78,96.	31,15,13, 13,19,23.	21,22,27, 28,51,18, 53.	24,26,29, 16,13,33.
	Zephyr	79,84,97, 142,86, 138,122, 104.	61,88,58, 105,72, 71.	10,18,25, 18,15,10, 20.	0,11,10, 11,6.	0,0,3, 0,0.
	Julia	98,99,87 113,121, 104,122.	86,95,68, 72,88,81, 74.	27,33,24, 22,46,24, 18.	35,44,25, 25,25,28, 9.	17,24,34, 16,25,53.
Leaves not pre-inoculated with P.hordei (conrtols)	Midas	122,141, 131,108. 87,84,79.	79,84,97, 136,134, 117,115.	32,19,24, 22,39,19.	70,63,55, 42,53,48.	82,77,51, 52,87,76.
	Zephyr	87,102, 69,142, 131,122.	86,57,96, 103,76,	42,33,26, 26,16,35.	45,45,46, 22,51,49.	94,98,103, 72,134, 140,107,98,
	Julia	90,80, 125,125, 113,130, 95.	71,75,99, 158,145, 146,105, 82.	36,46,33, 35,42,25.	26,24,41, 48,60,36.	74,94,96, 134,103, 126.

Table 38 Effects on rust of pre-inoculating leaves of four cultivars with E.graminis

		Mean no. rust pustules per leaf.				
Days between inoculations:		0	1	2	4	6
	Cultivar					
leaves pre-inoculated with E.graminis	Midas	290,21, 240,320, 190,180, 290.	5,52,67, 1,90,19, 10,36.	22,85,118, 56,136, 46,57.	103,52, 36,41,88,	27,0,14, 11,28,12.
	Zephyr	200,70, 270,150, 300,215.	88,58,27, 220,83.	13,88,63, 67,57.	22,48,67, 67,95, 230.	0,2,0, 1,0,0, 0.
	Julia	300,242, 250,261, 200,281, 198.	33,42,38, 76,72, 140.	68,81,34, 3,51,110,	53,124, 94,122, 105.	6,3,1, 6,0,0.
	Mazurka	200,130, 170,300, 250,140.	58,68,75, 64,230, 200,200.	3,42,64, 3,64,136.	172,14, 116,125, 150,1.	45,23,25, 76,5,22, 52.
Leaves not pre-inoculated with E.graminis (controls)	Midas	280,260, 340,210, 250,200, 200.	21,26,28, 31,15,38.	21,94,42, 128,8,46, 17.	98,250, 111.168, 85,156.	32,166 156,81, 53,152.
	Zephyr	210,240, 190,270.	52,82,95, 33,37,84, 24.	88,26,67, 144,120, 89.	84,162, 167,175, 104.	130,24, 109,124, 83.
	Julia	260,200, 200,230, 270,150.	56,133, 106,75, 51,154.	156,84, 242,155, 106,159, 113.	102,222, 230,150, 135,85.	96,136, 110,138, 166,197, 85.
	Mazurka	200,300, 270,300, 270.	123,20, 89,210, 98,131,180.	161,8,81, 1,40,194.	98,202, 196,216, 240,170.	85,93,62, 110,54,34.

Table 39 Dual infections of rust and mildew on four cultivars: effects on the development of rust and mildew of pre-inoculating leaves with E.graminis or P.hordei respectively.

Total area of lesions as percentage of the appropriate controls.

Days between inoculations:		Mildew colonies 0	1	2	4	6
	Cultivar					
Leaves	Midas	42.7	42.5	10.3	15.8	7.2
Pre-inoculated with	Zephyr	59.2	86.8	28.6	6.3	0.0
P.hordei.	Julia	80.5	36.7	26.6	25.9	1.2

		Rust pustules 0	1	2	4	6
	Cultivar					
Leaves	Midas	72.2	108.3	101.7	31.1	5.1
pre-	Zephyr	67.9	155.2	42.2	53.6	0.0
inoculated	Julia	141.7	46.7	17.5	41.6	0.0
with	Mazurka	46.41	96.3	47.6	33.1	22.7
E.graminis.						

Table 40 Dual inoculations of E.graminis and P.hordei on Zephyr barley: effects on mildew and rust of pre-inoculating leaves with E.graminis race B 71/98.

	P.hordei inoculated onto leaves		E.graminis inoculated onto leaves	
	pre-inoculated with E.graminis raceB71/98	not pre-inoculated	pre-inoculated with E.graminis raceB71/98	not pre-inoculated
	(A)	(C)	(B)	(D)
Rust pustules per leaf(A&C), mildew colonies per leaf (B&D)	94,144,134, 45,233,354, 215,42,144, 90,68,56.	88,135,168, 148,256,286, 187,229,146, 154,226,139.	5,8,3,6,4, 3,3,4,8,7, 8,5.	13,18,2,17, 4,8,18,35, 21,17.

Table 41 Dual inoculations of P.hordei and E.graminis on Mazurka: effect on rust of pre-inoculating leaves with E.graminis.

No. rust pustules per leaf.	Leaves pre-inoculated with E.graminis race(B71/98)	Leaves not pre-inoculated
	13,56,9,7,24,48,26,13, 24,38,39,63,45,50.	95,72,51,138,68,111,26,51, 12,20,12,74,142,69,65,63.
Means:	31.26*	66.81*

* Significantly different at $P \leqslant 0.01$

Table 42 Dual inoculations of P.hordei and E.graminis on Mazurka: effects of pre-inoculating leaves with E.graminis at varying periods before inoculations with P.hordei.

Days between inoculations:	Number of rust pustules per leaf on leaves; pre-inoculated with E.graminis.	not pre-inoculated
0	260,300,300,200,† 290,250,320,180, 190,250,260.	270,250,270,150, 100,100,180,150.
1	120,60,30,45,110, 60,30,40,130,70.	120,70,60,150,190,40, 170,110,200,160,70,60.
2	26,109,110,130,82, 25,63,115.	40,173,67,268,121,174, 221,246,153,81.
4	77,60,38,54,30,17, 30,22,64,78,55.	44,82,62,250,54, 30,40,140,39.

† Visual estimates were made where rust levels were high.

Table 43 Effect on rust of pre-inoculating leaves of Mazurka with E·graminis using different leaf surfaces.

	Leaves not pre-inoculated (control) (A)	Leaves pre-inoculated with E.graminis adaxial surface (B)	abaxial surface (C)
No. rust pustules per leaf.	28,81,66,89,67, 100,46,80,68.	46,75,52,25,70, 31,36,30,44.	85,26,45,26,12, 36,26,85,25,34, 45.
Mean:	69.4	45.4	40.3

Table 44 Effect on rust on second leaves of Mazurka, of pre-inoculating the first leaves with E.graminis.

	Plants not pre-inoculated with E.graminis. (control)	Plants with first leaves pre-inoculated with E.graminis.
No. rust pustules per leaf segment. (mean from 8-10 plants)	26.0,28.1,25.1, 11.4,30.0,19.4, 17.6,20.1.	30.6,24.9,15.2, 19.4,20.0,10.0, 22.1,9.5.
Mean(overall)	22.0	19.0

Table 45 Inoculation of first leaves of Mazurka with E.graminis followed immediately by inoculation with P.hordei.

	Leaves inoculated with P.hordei only	Leaves inoculated with P.hordei and E.graminis
No. of rust pustules (mean of ten fields 4.5mm diameter)	0.9,0.6,9.9,6.0,2.3, 6.4,3.1,5.0,9.5,1.8, 1.0,0.5,11.3,0.7,15.1, 10.3,19.7,5.5,3.7,2.1, 21.2.	14.6,12.3,10.5,0.7,5.2, 3.2,14.2,17.6,6.2,11.5, 1.0,1.1,8.6,10.0,15.6, 4.1,7.9.
Mean	6.505	8.489
No.per cm^2	40.91	53.39

Table 46 Inoculation of leaves with E.graminis: area of necrotic flecks and effects on the width of stomatal apertures.

	Days from inoculation with E.graminis.			
	4	6	8	12
Mean area of flecks (mm^2)	0.0259	0.135	0.216	0.279
Total area of flecks per mean leaf(mm^2)	1.67	8.70	13.75	17.96
% of total leaf area	0.23	1.19	1.88	2.45

Mean number of necrotic flecks per leaf = 64.3

Mean leaf area (cm^2) = 7.34

Width of stomatal apertures (μ)				
control leaves	1.003†	0.91	0.82	1.19
inoculated leaves	1.05	0.84	0.86	1.06

† mean of 200 stomata

No.rust pustules per leaf.

Leaves inoculated with P.hordei only(control)	Leaves pre-inoculated with E.graminis 4 days before inoculation with P.hordei
54,60,28,54,60,20,28, 18,30,36,57,82,42.	16,30,11,28,30,4,29, 22,12,12,6,12,44,12.
Mean: 43.0	19.14***

*** Significantly different from control at $P \leq 0.001$

Table 47 Effects on rust development of pre-inoculating leaves with P.hordei.

Number of rust pustules per leaf.

Treatments:	A	B	C	D	Control
	92,78,78, 48,34,39, 145,81,69.	20,25,88, 101,102, 35,60.	20,45,28, 68,64,75, 105.	16,30,31, 52,52,85, 42,15,60, 41,40.	174,48,45, 72,148,151, 119.

	Percentage uredospore germination(15 fields on 5 leaves) †	No.germ tubes reaching and entering stomata, with infection sites: present	absent
Leaves not pre-inoculated (control)	57.9	2, 1, 1, 3, 0, 2, 2, 0, 2, 3, 1, 3, 3, 1, 0.	2, 0, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 2, 0, 0.
Leaves pre-inoculated with E.graminis(treatment D above)	37.6	2, 0, 1, 2, 1, 1, 2, 2, 0, 0, 2, 0, 0, 0, 0.	3, 1, 0, 4, 1, 0, 1, 1, 0, 4, 1, 0, 0, 0, 0.

† = 300 - 400 uredospores

Table 48 Effects on rust of pre- inoculating leaves with E.graminis and of compounds likely to alter their carbohydrate content: using 2% sucrose solution

Size of rust pustules (mm^2)

Leaves not pre-inoculated and floated on :		Leaves pre-inoculated and floated on :	
water	2% sucrose	water	2% sucrose
.132,.166,.130,	.152,.193,.152,	.219,.080,.067,	.114,.422,.214,
.144,.218,.152,	.323,.214,.282,	.097,.122,.071,	.146,.163,.086,
.272,.265,.134,	.188,.152,.137,	.333,.072,.130,	.105,.155,.134,
.260,.207,.280,	.366,.332,.362,	.153,.090,.049,	.109,.141,.328,
.153,.146,.220,	.176,.190,.241,	.029,.095,.116,	.178,.160,.108,
.280,.190,.178,	.245,.349,.201,	.073,.102,.134,	.197,.118,.263,
.331,.245,.257,	.097,.137,.141,	.086,.133,.198,	.301,.087,.129,
.232,.130,.296.	.152,.331,.356.	.138,.213,.084.	.184,.247,.172.

Total chlorophyll content (mg. per litre)

2.62,	0.49	3.73	1.22
1.72	1.32	3.69	2.22
1.33	1.25	2.19	1.71

Table 49 Effects on rust of pre-inoculating leaves with E.graminis and of compounds likely to alter their carbohydrate content: using maleic hydrazide.

	Leaves not pre-inoculated		Leaves pre-inoculated	
	control	maleic hydrazide	control	maleic hydrazide
Rust pustules per leaf.	11,18,126,32, 24,36,58,44.	40,16,24,57, 24,18,58,38, 29,58,14,28, 21,30,16,13, 60,93,39,11.	28,41,33, 4,28,42, 28.	34,8,25,98,2, 23,72,46,12, 84,15,29,6,19, 18,18,23,18, 38,18,5.
Size of pustules (mm^2) mean of 10 pustules.	0.0676	0.0856	0.0677	0.0712
	0.0780	0.1037	0.0440	0.0677
	0.1042	0.0837	0.0496	0.0421
	0.0623	0.1035	0.0607	0.0608
	0.0890	0.1074	0.0572	0.0623
	0.0842	0.0956	0.0499	0.0545
	0.0714	0.1210	0.0938	0.0590
	0.0783	0.0896	0.0681	0.0673

Table 50 Inoculation of leaves with E.graminis: effect on the chlorophyll content and dry weight.

Days from inoculation:	Control leaves Fresh weight	Control leaves Chlorophyll mg/g D.wt.	Inoculated leaves Fresh weight	Inoculated leaves Chlorophyll mg/g D.wt.
0	0.431	22.71	0.454	21.70
	0.529	19.41	0.495	17.88
3	0.557	20.23	0.627	17.46
	0.541	24.35	0.493	20.62
6	0.630	19.11	0.548	16.62
	0.534	26.56	0.478	15.56
9	0.553	20.70	0.473	13.67
	0.590	25.65	0.510	15.79
12	0518	19.94	0.480	14.49
	0.528	23.54	0.471	12.31
15	0.487	22.73	0.484	10.42
	0.588	19.32	0.531	10.32
18	0.577	15.41	0.397	9.14
	0.638	15.42	0.315	8.51
21	0.488	12.57	0.205	3.24
	0.461	13.08	0.206	2.63
24	0.588	11.87	0.115	3.15
	0.452	14.08	0.061	1.69
29	0.428	7.79	0.034	2.09
	0.377	11.32	-	-
34	0.055	0.79	-	-
	-	-	-	-

Leaves inoculated with P.hordei

	No.rust pustules/leaf	Area of rust pustules(mm^2)mean/leaf
Control leaves	40,55,38,88,59,66,100, 148,75,108,112,56,94, 94,35,74.	.0334,.0656,.0642,.0959,.0683, .0791,.0709,.078,.0578,.052, .0725,.0689,.0587,.0697,.0499.
Leaves pre-inoculated with E.graminis.	22,24,57,85,15,48,57, 29,44,31,39,36,34,53, 49.	.049,.0504,.0472,.0446,.0485, .0424,.0424,.0468,.0408,.0397, .0544,.0272,.0553,.0476,.0292.

Table 51 Inoculation of Mazurka with E. graminis: effect on anti-fungal compounds.

Spore germination tests on E.graminis and P.hordei.

	Conidia	Uredospores
Distilled water control	39.8,42.9,38.8,37.0, 35.8,28.7,31.5,43.3, 31.9, 42.1.	34.3,26.6,29.8,22.9, 28.0,32.4,26.3,31.9, 37.8,35.0.

Extracts from leaves:

	uninoculated	inoculated	uninoculated	inoculated
Dilution 1	36.4	12.0	3.7	1.5
	26.2	19.9	2.8	3.8
	25.0	20.1	4.7	3.5
	20.4	18.4	3.2	3.9
	21.6	18.9	2.9	3.3
Dilution 2	31.0	26.5	4.8	12.7
	26.0	26.4	7.1	4.9
	18.3	22.5	8.6	6.2
	35.1	20.6	9.3	9.9
	33.3	18.5	7.0	6.5
Dilution 3	24.0	24.0	22.9	14.6
	35.9	28.8	22.3	15.4
	35.0	24.4	27.8	17.8
	27.9	27.6	14.7	20.7
	34.0	28.4	31.2	14.8
Dilution 4	32.4	27.7	20.6	18.8
	33.6	30.7	26.6	24.1
	28.2	31.1	25.9	20.5
	33.6	28.5	31.9	22.8
	39.3	25.0	29.1	23.3

Table 52 Inoculation of Mazurka with E.graminis: effects on antifungal compounds.

Extracts from uninoculated and inoculated Mazurka sprayed onto first leaves 6h prior to inoculation with:

Spray treatment used:	E.graminis		P.hordei	
	Colony no.	Area(mm^2)	Pustule no.	Area(mm^2)
Water and Tween 80 (control)	180,161,134, 91,121,125, 174,151, 75, 139.	2.78,1.97, 2.31,1.88, 2.38,2.33, 2.60,1.80.	90,60,89, 97,57,91. 66,48,62, 103.	0.076,0.085, 0.062,0.081, 0.092,0.077, 0.068,0.072,
Dilution 2 of extract from uninoculated.	34,57,74,81, 58,110,75, 50,182,131.	2.52,1.66, 1.39,2.04, 1.81,1.60, 0.83,2.01.	88,50,48, 68,38,88, 64,53,67, 79,78,81.	0.076,0.071, 0.062,0.101, 0.080,0.063, 0.054,0.085.
Dilution 2 of extract from inoculated leaves.	106,110,138, 90,46,95,54, 51,63,58,131, 35.	2.01,1.54, 2.56,1.36, 1.46,2.11, 1.58,1.65.	43,46,73, 54,94,78, 84,47,48, 126,58.	0.082,0.072, 0.099,0.077, 0.076,0.079, 0.067,0.064.
Dilution 3 of extract from uninoculated.	-	-	74,61,52, 50,81,72, 46,51,72, 28.	0.067,0.067, 0.073,0.061, 0.071,0.079, 0.098,0.096.
Dilution 3 of extract from inoculated leaves.	-	-	86,44,88, 68,44,42, 74,75,68, 84,85.	0.072,0.073, 0.072,0.062, 0.083,0.078, 0.087,0.114.

Table 53 Inoculation of Mazurka with E.graminis: effects on antifungal compounds 2.

Spore germination tests on E.graminis and P.hordei.

Treatment:	Percentage germination				
	Control distilled water	Extracts from leaves: uninoculated		inoculated with E.graminis.	
		aqueous	ethereal	aqueous	ethereal
Conidia of E.graminis	41.5	24.8	5.4	4.7	7.5
	41.8	49.2	10.8	11.0	8.9
	40.0	22.9	7.3	14.6	7.9
	31.9	38.2	9.9	12.6	12.8
	38.2	26.4	3.8	19.4	8.3
Uredospores of P.hordei	23.4	8.7	11.9	0.0	0.0
	19.5	5.1	12.3	1.6	0.0
	30.4	8.1	13.2	0.7	0.5
	26.1	2.5	1.8	0.9	0.6
	36.6	6.4	6.7	0.0	0.0

Each figure is a mean of at least 100 conidia or uredospores.

Table 54 Effects on rust and mildew of pre-inoculating leaves with Diplocarpon rosae or Uromyces phaseoli.

Non-pathogen used:	% cover of mildew/leaf†		Number of rust pustules/leaf	
	leaves not pre-inoculated	leaves pre-inoculated	leaves not pre-inoculated	leaves pre-inoculated
Uromyces phaseoli	40,10,15,15, 30,20,15,15.	30,20,15,15, 10,5,26,40, 15,5.	118,155,128, 144,109,133, 74,83,181.	77,71,71, 157,98,34, 204,134,145, 56,128,81, 47,19,75,74.
Means:	20.0	17.3	125.0	82.0

Table 54 (continued)

Non-pathogen used:	% cover of mildew/leaf		Number of rust pustules/leaf	
	Leaves not pre-inoculated	Leaves pre-inoculated	Leaves not pre-inoculated	Leaves pre-inoculated
Diplocarpon rosae	50,70,60,30, 30,40,55,65, 45,40.	40,40,70,40, 80,50,40,30, 25,15,25,15, 40,90,40,80.	20,54,10,75, 121,98,74, 61.	68,41,75,64, 87,71,94,79; 86,62,62,57, 151,10.
Means:	47.5	45.0	64.0	72.0

† visual estimates

Table 55 The effects of Calirus on the development of E.graminis

	Treatments: 1 Water only (control)	2 Calirus & Citowett	3 Citowett only	
Mildew development on treated plants; 6 days after inoculation.	92,48,69,56;† 85.	84,76,107,90, 48.	81,107,74,56, 93.	†mildew colonies/leaf
	3.61,3.08;†† 2.98,3.44, 2.66.	0.78,0.66, 0.721,0.27, 0.59.	0.64,0.50, 0.64.	††area of colonies(mm^2)
11 days after inoculation.	74,55,70,38,† 65.	57,43,54,37, 74.	58,63,62,56, 63.	†mildew colonies/leaf
	9.75,6.93;†† 10.12,8.32, 8.75.	4.40,3.41, 4.26,3.48, 2.75.	3.48,3.50, 2.31,3.45, 3.30.	††area of colonies(mm^2)
Mildew development on 'secondary plants' using treated leaves as sources of inoculum after :				
6 days	26,20,35,28, 46,40,45,40.	20,20,15,18, 16,28,26,16, 15,23.	19,21,31,17, 28,23,	
11 days	15,23,10,14, 15,11,32,19, 24,27,17,14, 18.	7,8,7,14,13, 8,15,14,10, 19,14.	15,8,6,10,15, 10,9,11,9,10, 8,7.	

Table 56 The effect of ethirimol on the development of P.hordei.

	Number of rust pustules per leaf on plants from:	
	single purpose dressed seed (no milstem) control	milstem dressed seed
	26,136,128,117,40,95,121, 90,69,167,85,94,70,46,75, 121,40,75,70,140,71,85,122.	175,112,71,166,102,110,67, 137,46,121,146,128,154,68, 72,65,88,78,111,140,74.
Means:	91.4	106.4

Table 57 The effect of ethirimol on the development of P.hordei on the third seedling leaf of barley.

The effect of milstem seed dressing and milgo spray on rust.

	P.hordei inoculated onto plants with:	
	no ethirimol treatment (control)	milstem dressed seed and milgo sprayed
No. rust pustules per field ($15.2mm^2$)	5.0,6.2,4.2,2.2,2.0, 5.8,1.6,0.8,0.4,1.4.	4.2,3.0,4.0,4.4,0.8, 1.4,3.8,3.2,3.0,3.6, 3.0,1.8,3.5.
Means:	2.76	3.05
Area of rust pustules(mean per leaf) (mm^2)	0.189,0.178,0.181, 0.145,0.135,0.132, 0.136,0.144,0.102.	0.157,0.154,0.166, 0.154,0.137,0.197, 0.141,0.147,0.129.
Means:	0.149	0.154

Table 58 The effect of ethirimol on the pustule productivity of rust.

	P.hordei inoculated onto plants treated with:		
	no chemical treatment (control)	milstem dressed seed	milgo sprayed
Mean no. rust pustules/leaf	154	157	156
	Mean weight per pot of uredospores collected (mg.)		
Days from pustule eruption:			
0	6.53	5.97	7.70
2	7.47	7.60	6.43
4	7.67	7.30	6.90
6	5.87	5.27	4.37
8	2.77	4.63	4.30
10	1.27	2.03	1.63

S E = $\pm$ 0.34

Table 59 The effect of ethirimol on the pustule productivity of rust.

P.hordei inoculated onto leaves from:

seed without milstem dressing			milstem dressed seed		
No.rust pustules per leaf	Wt.of spores(g) collected	Wt.of spores per pustule (mg)	No.rust pustules per leaf	Wt,of spores collected	Wt.of spores per pustule (mg)
175	0.0019	0.0109	194	0.0035	0.0180
244	0.0044	0.0180	185	0.0025	0.0122
120	0.0012	0.0100	104	0.0011	0.0106
110	0.0019	0.0173	86	0.0022	0.0256
118	0.0025	0.0212	106	0.0024	0.0222
44	0.0006	0.0136	98	0.0011	0.0112
114	0.0026	0.0228	70	0.0026	0.0364
42	0.0005	0.0119	112	0.0024	0.0210
84	0.0013	0.0155	44	0.0010	0.0227
75	0.0010	0.0133	86	0.0024	0.0279
Mean 112.6		0.0155	108.4		0.0208

Table 60 The effect of Calirus and ethirimol on barley yield in the absence of P.hordei and E.graminis.

Total dry weight of ears produced per plant(g.)

Treatment: 1 milstem dressed seed and ethirimol root drench.	2 plants sprayed with Calirus, (BAS3170F)	3 control, plants not chemically treated.
6.24,3.28,4.9,2.88,	5.02,6.43,8.11,	3.8,3.0,2.39,3.68,
5.11,2.32,3.43,3.7,	6.2,3.58,4.5,3.9,	3.2,4.05,4.5,3.62,
4.88,3.9,2.61,5.18,	5.73,3.1,5.3,5.1,	3.7,2.52,3.7,2.46,
4.6,3.04,2.47,3.3,	2.26,3.4,3.15,	4.24,3.6,3.11,3.5,
2.7,1.8,1.98,3.3,	2.3,2.6,1.5,3.7,	5.28,4.32,4.39,2.2,
2.8,2.8,4.88,3.88,	2.84,7.0,1.8,2.9,	5.18,10.8,3.12,2.22,
4.1,5.4,2.94,1.9,	4.2,3.0,3.42,7.3,	3.68,3.8,7.72,1.69,
3.19,7.7,3.3,3.92.	3.75,2.5,2.8,3.2,	4.19,3.16,2.1,1.91.
	2.86,3.72.	
Means: 3.700	3.975	3.682
Mean no. ears per plant: 3.26	3.28	3.13

Analysis of variance

Source of variation	Sum of squares	D.F.	Mean square	'F' test
Treatments	0.353	2	0.1765	0.069 n.s.
Replicates	60.065	31	2.0022	
Treatment/ replicates interaction	170.103	62	2.8351	
Error			2.5574	
Total	230.521			

Table 61 Development of mildew 1974.

Mean percentage cover per leaf for each plot

C.V.	Midas		Zephyr		Julia	
Treatment:	1	3	1	3	1	3
Date:						
4 June	2.25	4.01	7.29	9.08	3.54	8.01
	6.34	2.58	5.02	5.50	7.89	2.68
	5.02	2.85	14.47	16.06	15.61	8.17
11 June	2.94	3.50	5.55	6.58	4.55	5.04
	5.20	5.90	5.68	7.03	9.00	2.77
	4.20	5.13	21.63	22.82	10.68	9.03
18 June	10.18	13.72	21.84	11.50	25.85	26.00
	16.83	18.97	12.15	35.11	21.15	20.73
	13.06	11.02	32.17	28.92	29.99	13.70
25 June	11.41	12.15	36.18	20.44	30.42	23.58
	24.27	14.90	24.20	35.55	23.30	18.00
	18.71	16.46	37.83	33.52	30.90	36.35
2 July	25.13	25.60	34.63	35.81	42.25	33.92
	22.93	21.60	24.50	49.33	35.33	32.94
	29.07	30.60	42.10	41.25	27.78	49.33
9 July	18.69	25.31	36.60	45.34	39.79	28.69
	27.94	29.77	35.00	56.70	42.00	35.31
	31.56	35.63	55.75	56.17	44.61	41.88
16 July	21.17	21.33	33.94	39.08	43.28	51.83
	30.60	31.40	38.29	49.30	45.00	33.75
	32.18	27.90	51.19	56.39	34.75	44.60
19 July	25.45	29.42	42.14	45.28	49.43	41.04
	39.27	39.08	39.65	54.03	36.09	37.59
	25.88	32.81	54.86	49.30	42.36	42.11
23 July	26.14	29.12	35.00	29.00	38.54	44.00
	27.53	26.25	51.57	57.56	40.30	59.38
	11.21	15.44	40.42	34.88	29.10	36.63
26 July	6.57	8.38	47.25	14.69	52.00	45.00
	31.70	12.36	38.92	50.63	40.94	47.92
	16.99	20.28	42.50	15.00	35.00	28.13

Table 62 Development of mildew on flag leaves.

Mean percentage cover per leaf for each plot

Date:	2 July	9 July	16 July	19 July	23 July	26 July
Midas A	0.08	0.58	1.25	1.67	0.92	0.63
	0.00	0.00	0.17	0.42	0.50	2.17
	0.00	0.25	1.00	0.83	2.50	2.31
B	0.33	0.75	0.93	1.25	0.50	0.50
	0.00	0.33	7.50	10.08	6.33	4.17
	0.16	1.08	4.92	3.75	2.50	2.50
Zephyr A	4.33	5.58	7.83	15.58	15.83	30.50
	4.25	8.00	14.58	18.33	40.63	30.85
	0.08	0.83	7.50	8.33	8.75	7.50
B	5.35	5.92	11.67	15.42	12.50	8.13
	1.50	2.86	7.08	13.33	13.50	16.25
	0.00	3.33	12.92	13.33	13.50	15.00
Julia A	2.33	5.42	6.92	12.08	12.50.	19.00
	2.30	6.17	8.75	10.50	15.00	5.00
	0.16	0.25	6.75	17.08	10.50	6.25
B	2.17	7.33	20.5	28.75	25.50	37.50
	1.33	3.00	17.92	21.00	40.00	13.33
	5.58	7.33	10.50	15.08	18.75	15.00

A = Treatment 1 (mildew only)

B = Treatment 3 (mildew and rust)

Table 63 Development of mildew on leaf 2

Mean percentage per leaf for each plot

Date:	9 July	16 July	19 July	23 July	26 July
Midas A	7.92	10.08	16.67	15.00	12.50
	5.08	6.67	7.50	9.58	14.17
	12.75	17.83	19.17	19.92	21.67
B	10.92	15.17	21.25	18.10	16.25
	8.50	17.25	25.50	20.92	15.42
	13.75	15.58	17.92	18.00	20.83
Zephyr A	31.83	38.75	47.08	54.17	64.00
	28.08	35.80	45.00	62.50	35.00
	46.67	64.58	68.75	72.08	77.50
B	32.92	34.50	41.65	45.50	21.25
	50.71	58.33	62.50	69.17	85.00
	55.83	74.58	64.58	56.25	30.00
Julia A	38.33	42.08	51.25	64.58	85.00
	40.83	41.25	61.67	65.50	76.88
	30.83	37.50	50.00	48.41	63.75
B	28.42	45.00	55.33	62.50	52.50
	35.42	49.58	54.17	78.75	82.50
	40.83	43.30	48.75	54.50	41.25

A = Treatment 1 (mildew only)

B = Treatment 3 (mildew and rust)

Table 64 Development of mildew on leaf 3

Mean percentage cover of mildew, 3 replicates of 6 leaves.

Date:	Treatment 1 (mildew only)			Treatment 3 (mildew and rust)		
	Midas	Zephyr	Julia	Midas	Zephyr	Julia
11 June	0.22	4.64	2.89	0.11	2.58	7.27
18 June	3.92	24.50	37.60	4.45	19.83	35.40
25 June	11.97	47.2	37.60	10.90	39.90	54.80
2 July	28.50	58.60	66.10	29.30	65.80	73.00
9 July	39.20	64.90	73.00	38.90	74.60	74.60
16 July	41.40	63.30	75.30	44.90	78.40	85.00
19 July	58.30	68.90	72.50	57.20	78.40	62.50
23 July	67.50	-	-	48.40	90.00	-

Table 65 Development of mildew on leaf 4

Mean percentage cover of mildew, 3 replicates of 6 leaves.

Date:	Treatment 1 (mildew only)			Treatment 3 (mildew and rust)		
	Midas	Zephyr	Julia	Midas	Zephyr	Julia
4 June	0.34	1.22	0.70	0.16	0.75	0.39
11 June	8.30	14.10	11.67	8.70	16.70	12.20
18 June	24.10	46.40	51.30	24.00	48.30	49.20
25 June	27.10	62.30	67,50	38.30	69.90	79.20
2 July	52.00	60.00	85.00	53.30	78.20	80.00
9 July	50.00	52.50	-	56.10	85.80	-
16 July	48.30	-	-	53.00	-	-
19 July	58.00	-	-	62.50	-	-

- = leaves senescent

Table 66 Development of rust

Mean percentage cover of rust per leaf for each plot.

	Midas		Zephyr		Julia	
Treatment:	3	2	3	2	3	2
Date:						
2 July	0.00	0.00	0.00	0.17	0.00	0.04
	0.00	0.00	0.00	0.17	0.00	0.00
	0.00	0.35	0.00	0.17	0.00	0.00
9 July	0.00	0.00(YR)	0.00	0.93	0.00	0.23(YR)
	0.09	0.43	0.00	1.29(YR)	0.00	1.10(YR)
	0.10	0.91	0.10	3.13(YR)	0.00	0.27
16 July	0.46	1.15	1.32	6.13	0.17	0.55
	1.00	4.65	0.81	4.92	0.21	1.92
	1.02	4.46	0.78	6.94	0.52	1.81
19 July	2.77	4.73	2.08	6.38	0.67	3.11
	2.54	9.60	2.17	11.17	0.88	3.96
	2.61	9.52	1.70	18.33	1.22	4.38
23 July	2.34	4.78	2.08	11.12	0.15	2.61
	4.00	13.89	2.27	14.72	0.44	4.02
	4.50	15.39	3.20	27.84	2.08	5.50
26 July	5.77	9.21	5.00	10.94	1.12	3.88
	7.06	33.67	3.63	21.94	0.25	8.75
	10.56	26.00	5.25	31.88	4.50	7.35

YR = Yellow rust recorded present

Table 67 Early development of rust

Number of rust pustules (total for 6 plants per plot)

	Treatment 3 (mildew and rust)			Treatment 2 (rust only)		
Date :	25 June	2 July	9 July	25 June	2 July	9 July
Midas	0	12	68	0	14	115
	1	13	225	1	52	1000
	4	17	260	10	166	1555
Zephyr	0	1	78	18	93	1265
	3	12	143	5	95	1718
	0	10	255	20	192	4197
Julia	0	5	22	0	48	188
	0	1	7	0	19	522
	0	5	59	0	12	500

Analysis of variance to show cultivar differences

Treatment 3

Source of variation	D F.	S.S.	M.S.	'F' test	
Cultivars	2	15254.3	7627.15	3,5657	*
Date	2	86513.4	43256.20	20.222	**
Replicates	2	9534.4	4667.30		
Error	20		2138.90		
Total	26	166505.4		S.E. ± 15.41	

Treatment 2 (rust only)

Source of variation	D.F.	S.S.	M.S.	'F' test	
Cultivars	2	1159498.3	579749.5	5.8332	**
Date	2	5868214.7	2034107.4	29.5216	**
Replicates	2	537325.4	268662.7		
Error	20		99388.4		
Total	26	10651484.5		S.E. ± 32.25	

Table 68 Development of rust on flag leaves.

Mean percentage rust per leaf for each plot at each assessment date.

Date:	9 July	16 July	19 July	23 July	26 July
Midas	0.00	0.42	2.42	4.17	6.40
A	0.00	0.83	2.25	4.58	5.17
	0.00	1.33	3.75	5.58	9.17
B	0.00(YR)	0.33	1.92	4.90	8.13
	0.00	1.83	5.50	7.08	18.50
	0.00	1.42	6.83	10.75	15.00
Zephyr	0.00	1.08	2.33	2.67	4.25
A	0.00	1.17	3.00	3.30	2.25
	0.00	1.00	2.92	2.90	5.00
B	0.00	0.58	2.17	4.33	8.25
	0.00	1.83	4.67	9.17	10.83
	0.00	2.75	10.83	16.67	23.75
Julia	0.00	0.17	0.58	0.30	2.25
A	0.00	0.25	1.17	0.75	0.50
	0.00	1.00	1.42	2.25	5.00
B	0.00(YR)	0.33	1.75	0.92	1.50
	0.00	0.08	1.17	1.00	13.75
	0.00	0.08	0.92	1.90	2.90

A = Treatment 3 (rust and mildew)

B = Treatment 2 (rust only, mildew controlled)

YR = Yellow rust present

Table 69 Development of rust on leaf 2(flag leaf=1)

Mean percentage rust per leaf for each plot on each assessment date

Date:	9 July	16 July	19 July	23 July	26 July
Midas	0.00	1.17	4.00	2.60	5.13
A	0.00	1.17	3.25	6.42	8.50
	0.17	1.00	3.92	6.25	10.00
B	0.00	0.67	3.83	3.83	7.00
	0.17	3.33	10.83	14.58	32.50
	0.33	3.50	10.00	16.67	33.00
Zephyr	0.00	1.58	2.67	1.50	5.75
A	0.00	0.92	2.50	2.50	5.00
	0.50	0.83	2.17	3.50	5.55
B	0.00	1.75	6.33	17.92	13.13
	1.00	4.50	13.33	18.75	25.00
	2.33(YR)	6.50	21.67	39.00	40.00
Julia	0.00	0.34	0.75	0.00	0.00
A	0.00	0.17	0.58	0.18	0.00
	0.00	0.42	1.75	1.90	4.00
B	0.00	0.86	3.08	2.67	6.25
	0.00(YR)	1.08	3.67	4.17	13.50
	0.17	2.50	4.50	4.60	11.30

A = Treatment 3 (rust and mildew)

B = Treatment 2 (rust only, mildew controlled)

YR = Yellow rust present

Table 70 Development of rust on leaf 3

Percentage cover of rust, mean of 3 replicates each of 6 leaves.

	Treatment 3 (rust and mildew)			Treatment 2 (rust only, mildew controlled)		
Date:	Midas	Zephyr	Julia	Midas	Zephyr	Julia
2 July	0.00	0.00	0.00	0.17	0.17	0.17
9 July	0.33	0.00	0.00	1.76	4.17(YR)	0.50(YR)
16 July	0.98	0.71	0.04	5.00	10.61	2.14
19 July	7.27	0.75	0.50	10.96	16.67	5.8
23 July	0.97	1.00	-	14.72	16.25	6.7

- Leaves senescent

Table 71 Development of rust on leaf 4

Percentage cover of rust, mean of 3 replicates each of 6 leaves.

	Treatment 3 (rust and mildew)			Treatment 2 (rust only mildew controlled)		
Date:	Midas	Zephyr	Julia	Midas	Zephyr	Julia
2 July	0.00	0.00	0.00	0.03	0.00	0.00
9 July	0.03	0.00	-	0.74	2.18	0.36
16 July	0.50	0.00	-	4.09	5.83	2.88
19 July	1.25	-	-	8.14	5.00	5.25

- Leaves senescent.

Table 72 Development of rust on leaf 2

		Mean no. pustules per cm^2 [a]	Mean area of pustules (mm^2) [b]
Midas	A	67.1, 92.1, 132.9	0.04, 0.034, 0.037
	B	104.7, 241.8, 208.2	0.043, 0.043, 0.048
Zephyr	A	113.7, 144.9, 106.6	0.035, 0.032, 0.036
	B	151.3, 231.3, 236.2	0.050, 0.041, 0.045
Julia	A	55.2, 41.6, 80.5	0.034, 0.028, 0.029
	B	91.8, 89.7, 117.1	0.038, 0.040, 0.036

A = Treatment 3 (rust and mildew) B = Treatment 2 (rust only)

[a] = Values are means of 25 recordings

[b] = Values are means from 50 pustules

Table 73 Disease development on late tillers

	Mean % cover of mildew on flag leaf†		Mean % cover of rust on flag leaf†	
Treatment:	1 (mildew only)	3 (mildew and rust)	3 (mildew and rust)	2 (rust only)
Midas	6.00	0.17	20.8	30.0
	10.00	0.00	29.0	38.3
	4.50	0.00	26.0	31.0
Zephyr	8.63	0.20	10.0	14.0
	2.70	4.00	7.1	9.0
	2.70	2.90	8.5	19.1
Julia	7.50	2.30	2.1	12.0
	5.00	4.00	7.4	15.1
	6.50	2.50	10.7	18.7

† Mean from 5 plants

Table 74 Field Trial 1974 Silwood Park.

Yield of grain per plot (Kg)

(corrected for % moisture content)

Treatment:	Mildew only	Rust only	Mildew and rust	Neither disease
Midas	2.51	2.25	2.19	2.78
	3.04	3.08	2.81	3.76
	3.08	2.61	3.11	3.26
Zephyr	1.24	2.21	1.62	2.38
	2.17	2.39	2.10	2.61
	1.51	2.33	1.49	2.35
Julia	2.19	2.14	1.57	2.26
	2.33	2.36	2.70	2.85
	2.02	2.88	2.17	3.02

Mean percentage yield losses due to rust and mildew

Midas	12.54	18.96	17.10	—
Zephyr	33.03	5.71	28.98	—
Julia	20.70	9.23	20.70	—

Analysis of variance: Plot yields 1974

Source of variation	D.F.	Sum of squares	Mean square	'F' test
Treatments	3	2.143	0.7142	10.17***
Cultivars	2	4.269	2.1344	32.19***
Blocks	2	2.021	1.0106	15.24***
Cult.X Treatments	6	0.657	0.1095	1.65 n.s.
Error	22	1.458	0.0663	
Total	35	10.548		

Table 74 (continued)

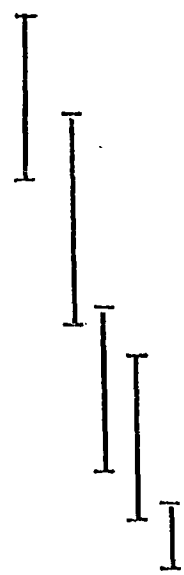
Further analysis Plot yields 1974

Analysis of variance - Treatments

Source of variation	D.F.	Sum of squares	Mean square	'F' test	Probability
Mildew	1	1.6329	1.6329	24.63***	0.1%
Rust	1	0.3071	0.3071	4.63*	5%
Mildew x rust	1	0.2027	0.2027	3.05 n.s.	10%
Error			0.0663		
Total	3	2.1426			

Duncans Multiple Range Test. (5%)

Treatment	Mean yield(Kg)
Zephyr mildew only	1.641
Zephyr mildew + rust	1.739
Julia mildew + rust	2.147
Julia mildew only	2.176
Zephyr rust only	2.311
Zephyr neither disease	2.445
Julia rust only	2.460
Midas rust only	2.646
Midas rust + mildew	2.705
Julia neither disease	2.711
Midas mildew only	2.876
Midas neither disease	3.265



Analysis of variance - Treatments of each cultivar.

Source of variation	D.F.	'F' values Midas	Zephyr	Julia
Mildew	1	1.97	7.28*	78.96***
Rust	1	11.16*	0.02	0.59
Mildew x rust	1	3.53	0.26	0.04
Blocks	2	14.37**	0.11	6.69*

Values significant at $P \leq 0.05$; 0.01; and 0.001 denoted by *, **, and *** respectively

Table 75 Field Trial 1974 Silwood Park.

1,000 grain weights (g.) per plot.

Treatments:	Mildew only	Rust only	Mildew and rust	Neither disease
Midas	32.33	31.53	31.97	36.50
	26.17	31.23	31.17	36.33
	30.07	30.50	28.73	35.17
Zephyr	35.57	36.10	33.90	38.77
	31.20	34.80	31.10	38.43
	33.37	33.47	31.03	38.97
Julia	31.27	33.80	33.63	41.23
	34.77	37.70	37.00	38.70
	33.03	36.00	34.47	41.63

Analysis of variance

Source of variation	D.F.	Sum of squares	Mean square	'F'-Test	Probability
Treatments	3	76.183	28.092	52.00	0.1 %
Cultivars	2	35.1177	17.559	32.50	0.1 %
Error	6	3.2419	0.540		
Total	11	114.543			

Further analysis, separation of Treatment variation

Mildew	1	48.2804	48.2804	89.358	0.1 %
Rust	1	10.2121	10.2121	18.9008	1.0 %
Mildew x rust	1	17.6904	17.6904	32.7418	1.0 %

Treatment variation on individual cultivars.

Source of variation	'F' values	Midas	Zephyr	Julia
Mildew		73.227***	41.137***	19.012**
Rust		22.109***	17.533**	1.979
Mildew x rust		54.977***	4.096	10.407*
Blocks		9.897*	4.696	1.599

F values significant at $P \leqslant 0.05$, 0.01 and 0.001 denoted by *, **, and *** respectively.

Table 76 Sieve tests on dried grain.

Weight of grain retained by each sieve

(Mean of 2 replicates from 3 plots)

Treatment:		Mildew only	Rust only	Mildew and rust	Neither disease
Sieve size(mm)					
Midas	2.8	6.98	6.08	5.75	8.02
	2.5	41.14	35.37	40.47	40.81
	2.2	34.18	35.94	33.31	32.04
	< 2.2	17.69	25.94	19.14	19.12
Zephyr	2.8	14.51	11.95	12.01	14.61
	2.5	40.63	38.40	40.24	41.98
	2.2	27.69	27.57	28.46	25.19
	<2.2	17.17	22.07	19.30	18.27
Julia	2.8	11.52	18.00	14.09	22.54
	2.5	42.64	42.63	43.00	43.21
	2.2	29.13	23.50	28.03	20.83
	< 2.2	16.71	15.87	14.89	13.43

Analysis of variance: Weight of grain in sieves 1 and 2(2.8 & 2.5mm) summed.

Source of variation	D.F.	Sum of squares	Mean square	'F'-Test	Probability.
Treatments	3	68.5066	22.836	2.176	n.s.
Cultivars	2	352.9695	176.485	16.815	1.0%
Treat x cult.	6	62.9758	10.496		
Error			10.496		
Total	11	484.4520			

Further analysis, separation of treatment variation.

Mildew	1	9.398	9.398	0.895	n.s.
Rust	1	35.365	35.365	3.369	n.s.
Mildew x rust	1	23.744	23.744	2.262	n.s.

Table 77 Field Trial 1975, disease assessments.

Mean % cover of mildew per leaf, July 1975.

Treatments:	Mildew only			Rust only			Mildew and rust			Neither disease			
Date:	7	14	21	7	14	21	7	14	21	7	14	21	July
Midas	2.67	4.50	5.80	0.0	0.14	0.00	5.9	15.9	2.8	0.4	0.07	0.5	
	3.9	8.2	4.9	0.5	1.8	0.4	12.6	22.8	29.9	0.1	1.2	1.0	
	12.6	38.4	20.4	3.8	11.8	5.7	2.9	8.5	1.0	0.0	0.2	0.0	
Zephyr	21.8	41.3	25.5	0.2	0.7	0.0	13.6	14.7	25.1	0.3	0.3	2.0	
	31.9	49.6	21.9	1.0	4.1	0.3	5.6	39.3	28.4	0.6	4.9	0.0	
	14.1	37.2	23.2	13.7	35.7	19.7	19.8	38.2	26.5	0.1	5.0	0.0	
July	5.5	13.8	19.3	1.1	3.9	1.9	11.7	27.2	15.1	0.5	1.96	0.0	
	4.6	10.2	2.8	0.1	0.3	3.1	19.5	29.6	18.6	2.0	5.7	0.0	
	11.9	31.3	27.9	0.9	2.0	9.7	12.5	42.5	25.2	0.0	1.1	1.0	

Analysis of variance.

Source of variation	'F' values			
Date:	7	14	21	July.
Presence of rust	0.039	0.007	0.276	
Cultivar	5.090*	4.519*	3.451	
S.E.±	3.483	6.763	5.445	

Variance ratios (F) significant at $P \leq 0.05$ denoted by *.

Table 78 Field trial 1975

Mean rust pustule no.per leaf on 7 and 14 July;

mean % cover on 21 July.

Treatments:	Mildew only			Rust only			Mildew and rust			Neither disease		
Date:	7	14	21	7	14	21	7	14	21	7	14	21
Midas	1.5	0.33	0.5	0.7	1.44	0.17	0.83	0.13	0.42	0.40	0.23	0.5
	0.5	0.4	0.08	1.8	0.73	1.92	0.07	0.23	1.16	0.1	1.0	0.5
	0.1	0.5	0.73	1.6	1.6	5.36	0.09	0.22	2.3	1.1	0.61	0.0
Zephyr	0.0	0.06	0.0	1.0	0.07	1.6	0.0	0.13	0.15	0.17	0.06	0.5
	0.0	0.06	0.06	2.7	1.3	5.68	0.4	0.23	1.33	0.4	0.07	0.0
	0.0	0.33	0.13	0.4	1.7	4.2	0.17	0.33	0.71	0.17	0.30	0.0
Julia	0.16	0.0	0.0	0.2	0.05	0.32	0.1	0.0	0.0	0.61	0.17	0.5
	0.11	0.11	0.0	0.1	0.0	2.25	0.11	0.0	0.12	0.22	0.2	0.5
	0.25	0.1	0.16	0.6	0.06	0.06	0.17	0.06	0.0	0.3	0.4	0.5

Table 79 Spore Trap Data 1975.

Total spores collected over three weeks,

7 to 28 July.

Treatment:	Mildew only		Rust only		Mildew and rust		Neither disease.	
	A	B	A	B	A	B	A	B
Midas	32	740	96	480	108	480	76	292
	54	606	124	404	152	612	64	536
	64	505	96	372	116	236	68	428
Zephyr	64	658	84	380	52	344	48	260
	56	468	104	248	56	620	32	376
	124	388	224	372	36	420	28	132
Julia	48	308	44	220	12	416	40	212
	64	598	36	244	32	172	48	268
	16	132	236	256	20	184	32	144

Analysis of variance		A. Uredospores			B. Conidia		
Source of variation	DF.	Sum of squares	Mean square	'F' test	Sum of squares	Mean square	'F' test
Treatments	3	24635.9	8211.9	4.68*	128179.6	42726.5	2.416 n.s.
Cultivars	2	7684.7	3842.3	2.19	211418.0	105709.0	5.98*
Blocks	2	5480.7	2740.3	1.56	172442.0	86221.0	4.875*
Treat x cult.	6	16389.1	2731.5		36517.1	6086.2	
Error	22		1754.3			17687.8	
Total	35	92786.9			937687.9		

Separation of treatment variation;		Uredospores.			Probability
Mildew	1	3885.4	3885.4	2.215 n.s.	20%
Rust	1	6029.4	6029.4	3.437 n.s.	10%
Mildew x rust	1	14721.0	14721.0	8.391**	1%
Error		1754.3			

* values significant at $P \leq 0.05$.

Table 80 Field Trial 1975 Spore trap data.

Treatment:	Mildew only		Rust only		Mildew and rust		Neither disease	
7 - 14 July	Uredospores	Conidia	Uredospores	Conidia	Uredospores	Conidia	Uredospores	Conidia
Midas	20	420	24	248	0	140	16	240
	36	240	20	348	24	228	8	224
	4	120	32	192	4	180	16	288
Zephyr	0	440	12	204	8	172	32	168
	0	248	8	308	12	212	32	88
	28	120	12	184	4	72	16	200
Julia	8	196	0	260	4	120	0	124
	32	378	4	80	0	180	8	100
	0	100	0	88	4	84	36	144
14 - 21 July								
Midas	8	132	32	108	24	40	24	128
	12	140	36	140	8	188	4	120
	12	40	48	28	24	148	40	56
Zephyr	8	126	4	76	4	44	24	152
	44	180	44	92	8	44	16	124
	40	152	12	136	4	28	16	100
Julia	4	20	0	100	8	52	28	48
	24	120	4	48	12	40	12	56
	0	20	4	56	8	32	140	72
21 - 28 July								
Midas	4	188	52	124	52	112	56	112
	8	224	96	124	32	120	112	60
	48	12	36	16	40	100	40	28
Zephyr	56	92	36	64	36	44	28	60
	12	40	4	220	12	120	56	36
	56	116	12	100	20	32	192	72
Julia	36	92	12	56	28	40	16	48
	8	100	24	44	36	48	16	88
	16	12	16	40	20	28	60	40

Analysis of variance		Uredospores 'F' values			Conidia 'F' values		
Source	July	7-14	14-21	21-28	7-14	14-21	21-28
Treatments		1.667	1.536	2.256	1.899	1.164	0.892
Cultivars		1.996	0.090	1.875	2.479	5.752**	2.659
Blocks		0.605	1.328	0.417	2.680	2.223	3.172

Value significant at $P = 0.01$ indicated by **

Table 81 Field trial 1975.

Yield (Kg) per plot corrected for % moisture content.

Treatments:	Mildew only	Rust only	Mildew and rust	Neither disease
Midas	1.80	2.86	2.52	4.22
	3.54	4.19	3.22	3.77
	4.13	4.28	1.72	3.88
Zephyr	1.74	2.00	1.29	2.71
	2.40	2.63	1.88	2.46
	1.43	2.87	1.54	2.64
Julia	2.56	3.48	2.71	2.93
	2.88	2.41	2.20	2.99
	2.44	2.42	2.35	2.82

Table 82 1,000 grain weights(g.), mean per plot.

Treatments:	Mildew only	Rust only	Mildew and rust	Neither disease
Midas	34.4	35.7	34.8	35.1
	35.8	35.3	34.3	36.1
	34.8	35.3	34.3	35.1
Zephyr	35.8	35.7	33.4	36.3
	34.1	36.0	34.9	35.1
	35.0	34.3	34.2	35.5
Julia	35.4	38.2	36.9	37.8
	37.1	37.2	36.3	37.0
	35.9	38.8	34.7	37.4

Addendum.

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