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An ecophysiological approach to crop losses exemplified in the system wheat, leaf rust and glume blotch

II. Development, growth, and transpiration of uninfected plants and plants infected with Puccinia recondita f.sp. triticina and/or Septoria nodorum in a climate chamber experiment

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Abstract

A climate chamber experiment is reported in which were investigated the growth and transpiration of uninfected wheat plants (C), plants infected with either *Puccinia recondita* (leaf rust) alone (R), *Septoria nodorum* (glume blotch) alone (S), or with both pathogens together (I). The rust inoculation was at the 75% heading stage, and was followed four days later by the glume blotch inoculation; re-infection was prevented. Effects of disease on axial development were not observed. The rate of total dry weight increase of the plants was reduced in S and I, mainly because of smaller dry weight increase of the heads. Kernel weight and kernel number in I were lower than in C, R, and S. Stems in S and I were shorter than those in C and R, and their weights were lower. Rapid root deterioration was observed in I. The transpiration was greater in R than in C, in S smaller. Transpiration in I was initially equal to that in R, but the transpiration rate decreased rapidly after the glume blotch symptoms became visible. The increase in the percentage of infection by rust in I was lower than in R, and the sporulation came almost to a stop soon after the appearance of glume blotch flecks. The percentage of infection by glume blotch in I increased faster than in S. Regression equations for growth and transpiration are given.

Introduction

The experiment described here on the effects of infection by pathogens on the yield of plants was performed to gain an understanding of the causes of loss in field crops. Losses are determined at harvest time. An effective method of gaining information about the process that leads to loss is to study the physiology of infected plants from infection to harvest. It is necessary to know the physiological state of the plant at the time of infection, as the response to infection, and consequently the loss, may be related to this physiological state. As the state of the plant at any time is the outcome of its total previous 'history', it is necessary to record the history of the plant from the seedling stage to the time of infection.

Yield and loss are functions of host, pathogen and environment. An infinite number of combinations of cultivars, pathogens and environmental factors can be made. The study of the ecophysiology of crop losses by experiment alone is almost impossible. A feasible approach is the balanced combination of experiment with other techniques

such as dynamic simulation. Before this 'balanced' combination can be considered, the methodological and organizational aspects of crop loss experiments must be established. Indoor experiments can assist in the development of concepts and methods, and they provide information that is useful in the interpretation of results obtained under field conditions.

This paper discusses the methodology and the organization of experimental research into the ecophysiology of uninfected wheat plants, and wheat plants infected with either *Puccinia recondita* (Rob. ex Desm.) f.sp. *triticina* Eriks. (leaf rust) or *Septoria nodorum* (Berk.) Berk. (glume blotch), or both pathogens together.

Methodology

General remarks

A separate treatment of principles, practices and procedures is necessary because of the diversity of the subject. This section considers terminology, choice of experimental and situational factors, choice of responses, selection of sampling techniques, and the choice of statistical methods.

Terminology

Loss is here defined as the difference in yield between infected and uninfected plants, grown under identical conditions. Loss is measured at harvest. Only the yield of grain is taken into account. Loss is expressed in units of mass. Specific loss is expressed in units of mass per plant, or per unit of area.

Injury is defined as the deviation of the physiology and/or appearance of the treated plant from the corresponding properties of the untreated plant, where the treatment may be either infection by pathogens or change of the environment, under otherwise identical conditions. Pathogens and environment can both act as injurious agents.

Physiology. In this paper, the concept is limited to the processes of development, growth, and transpiration.

Growth is defined here as a change in the state of the plant that can be expressed quantitatively such as length, area, volume, or weight. Growth rate is growth per unit time.

Transpiration and evaporation. In this paper, transpiration is the loss of weight of a plant due to water loss, and evaporation that of the soil due to water loss, expressed in units of mass. Specific transpiration is expressed in units of mass per plant or per unit of area.

Symptoms. The difference in appearance between infected and uninfected plants is determined by many phenomena or factors, some of which are typical of one particular pathogen-host combination, and others are not. Both the typical and the atypical effects of the pathogen(s) on the appearance of the host plant are here indicated by the term symptoms.

Variables, experimental and situational factors

Any factor that can be varied in an experiment is termed a variable. In most experiments, only a few variables are set at more than one level in the experimental design, and these are termed experimental factors. The remaining variables, appearing only at one level, can be referred to as situational factors (Zadoks, 1972). The results of ecophysiological experiments are largely determined by the choice of the levels at which the situational factors are set. Only one spring wheat cultivar, 'Kolibri', and one isolate of each of the pathogens were used. Only one inoculation with each pathogen at one spore density was carried out. The various combinations of diseases can be considered as different levels of one variable, viz. 'disease treatment': C = uninfected control plant; R = plant with rust alone; S = plant with glume blotch alone; I = plant with both rust and glume blotch.

Responses

In this single input – multiple output experiment (Zadoks, 1972), a number of output variables, here called 'responses', was assessed. The number of responses monitored was limited owing to restrictions of manpower and equipment. After inoculation, growth and development of the pathogens were described too. The following responses were selected.

Development. Growth stages according to Zadoks et al. (1974). Axial development according to Schoute (1910).

Growth. Dry weights of shoots, roots, leaves (including mycelium in infected leaves), stems with leaf sheaths, heads and kernels; number of kernels; leaf area.

Growth and development of the pathogens. Part of the plant infected and percentage of infection; time of appearance of first uredosori, teleutosori, flecks and pycnidia.

Miscellaneous. Where possible, other responses such as length and width of leaves, length of stems or internodes, number of leaves per axis, and temperature of the plant organs (stems, heads, leaves) were determined.

Experimental design and sampling

The smallest experimental unit was the single plant with the soil in which it was growing. Six plants together in one bucket formed the measuring unit for the transpiration measurements. Four buckets, containing 24 plants, were subjected to one level of disease treatment. Transpiration measurements were obtained for each of the four buckets belonging to each group. Treatments and replicates were randomized over the experimental area of the growth chamber. The arrangement of the experiment (Fig. 1 and 2) in the climate chamber provided an inner 'experimental area' of 16 buckets with a border of 20 buckets. In the horizontal plane, steep environment gradients existed at the edge of the 'crop', but in the experimental area the gradients were mild. The buckets were placed close together on three trolleys which could be

Fig. 1. Cross-section through the climate chamber showing the pattern of air flow (dotted lines with arrow-heads) and the position of plants, lamps, balance, and masts with sensors. T = temperature sensor. RH = air humidity sensor. Heights above soil level in the buckets (Bu) are given in 10^{-2}.m. Levels 1, 2 and 3 indicate the three positions of the buckets successively taken during the experiment. B = balance for transpiration measurements. HPLR 400 = high pressure mercury vapour lamp 400 W. TL = TL 40 W tubular lamps.

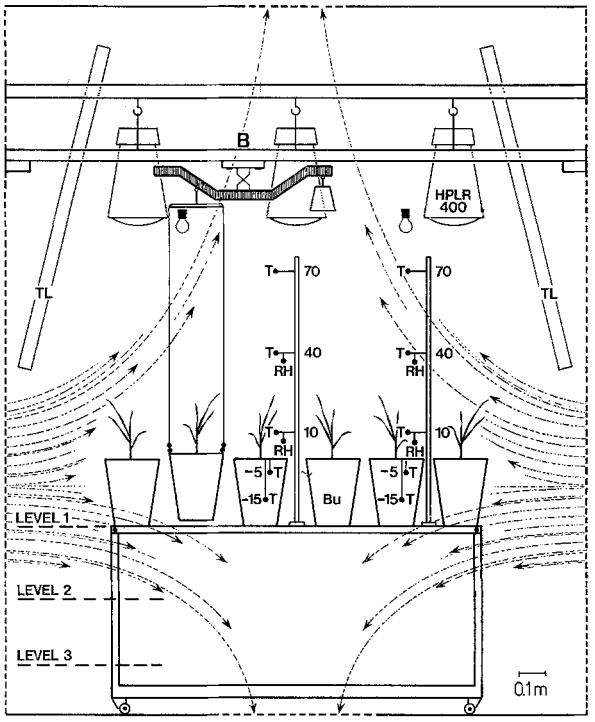

Fig. 1. Dwarsdoorsnede door de klimaatkamer met de stroomrichting van de lucht (gestippelde lijnen met pijltjes) en de plaats van planten, lampen, balans en masten met meetvoelers. T = temperatuurvoeler. RH = voeler voor luchtvochtigheid. De hoogte van de meetvoelers t.o.v. het grondniveau in de emmers (Bu) is aangegeven in 10^{-2}.m. Niveaus 1, 2 en 3 duiden de drie hoogten van de emmers aan, achtereenvolgens ingenomen gedurende de proef. B = balans voor transpiratiemetingen. HPLR 400 = hoge druk kwiklamp 400 W. TL = TL fluorescentie lampen 40 W.

moved to facilitate watering and sampling.

Some plants in the border buckets near the long axis of the chamber (Fig. 2) were destructively sampled in the period before inoculation. After inoculation, 16 plants were sampled (four per disease treatment) at one-week intervals. The four plants per treatment were taken at random from two buckets. The sampling of plants for the determination of dry weight and leaf area commenced four weeks after sowing. In the period before inoculation only three plants per sample were taken; after inoculation, a sample consisted of four plants per treatment (16 plants per sample). Results are presented in terms of the mean values per plant.

Transpiration was measured continuously using three pivot spring balances (Tegelaar and Van der Wal, 1974). The transpiration calculated during the first half of the growing period was based on 3 buckets containing 6 plants each, each of them being

Fig. 2. Projection on a horizontal plane in the climate chamber. The places of the buckets are indicated by rectangles: those with a diagonal represent buckets of the 'border', those without a diagonal constitute the 'experimental area'. In one rectangle, the six bags with one plant each are indicated. The diagram shows the positions of TL lamps (TL) along the perforated walls and of the HPLR lamps above the buckets (dotted circles). CS = position of sensors of the control equipment. The multipoint plug is used in signal transport from the sensors to the recorder room. A, B, C, and D indicate positions of masts with sensors. B1, B2, and B3 = position of the three buckets hanging from the balances 1, 2, and 3.

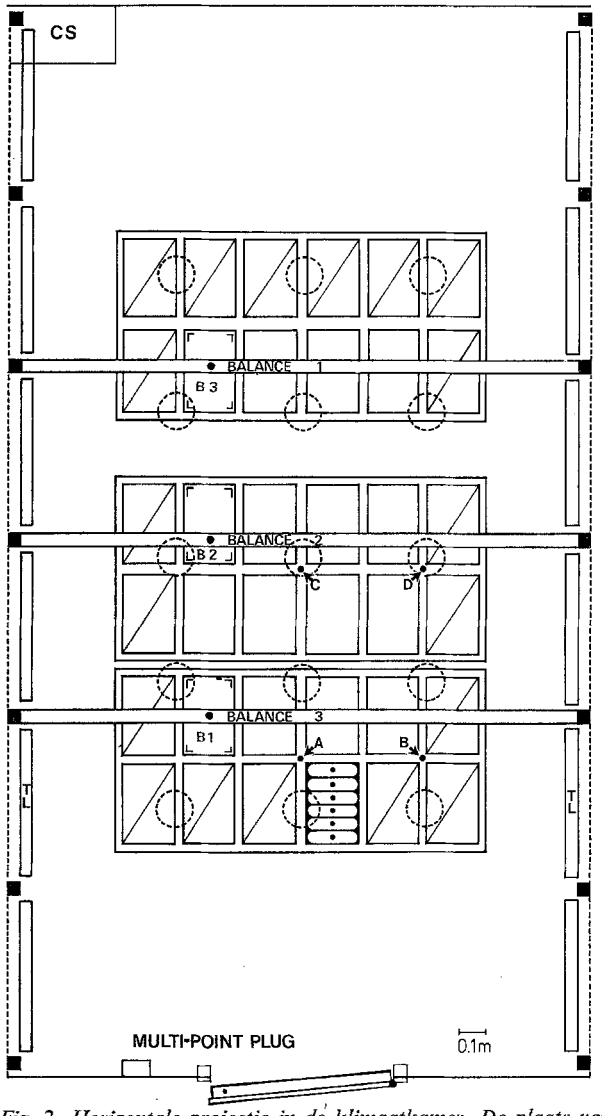

Fig. 2. Horizontale projectie in de klimaatkamer. De plaats van de emmers is aangeduid met rechthoeken; die met een diagonaal duiden emmers aan die tot de 'rand' behoren, die zonder diagonaal vormen het 'proefgebied'. In één emmer zijn de zes zakken met elk een plant aangeduid. De plaatsen van de TL-buizen bij de muren (TL) en die van de HPLR-lampen (gestippelde cirkels) zijn eveneens aangegeven. CS = voelers van de regelapparatuur van de klimaatkamer. De meerpuntsplug wordt gebruikt bij het overbrengen van de signalen van de meetvoelers naar de recorderkamer. A, B, C en D geven de plaatsen van de meetmasten aan. B1, B2 en B3 = plaatsen van de drie emmers aan de balansen 1, 2 en 3.

on the same balance in this period. The 3 balances allowed of the monitoring of transpiration in 21 buckets during one week, and after inoculation a rotation scheme was applied so that simultaneous measurement of more than one replicate in any treatment was avoided as much as possible. A correction factor for evaporation was calculated from the results of measurements during the first four days of the growth period, when the first leaf was emerging. A mean specific correction factor of 30.1×10^{-3} kg. day^{-1}. $plant^{-1}$ (strictly, per soil bag) was obtained. For each treatment, an estimate of the daily transpiration was made by linear interpolation of the original transpiration data.

Statistical methods

Responses like leaf area and dry weight of individual plants show great variance. In view of the limitations of available space and labour, a choice had to be made between either just a few large samples, or more but smaller samples. If a few large samples are used the information about the state of the plant at sampling date is relatively accurate but little insight is gained into the growth process itself. In this approach, called cross-sectional approach (Zadoks, 1972), emphasis is on the best possible estimate of the state of the plant; analysis of variance is a suitable technique to detect significant differences between the levels of disease treatment. In the present paper, where emphasis is on the process itself, a longitudinal approach is more appropriate. Consequently, other statistical methods have to be used in order to detect differences between effects of the various levels of disease treatment during a certain period. As the longitudinal approach necessitates frequent sampling, the choice fell on taking small samples: four plants per level of disease treatment and per sampling date, and six sampling dates in the period from inoculation to harvest.

The first step analysing the data was a test of the significance of the differences between the response curves corresponding with the four levels of disease treatment. All six possible combinations of two levels of disease treatment (C-R, C-S, C-I, R-S, R-I, and S-I) were studied. One other combination, C-R-S+I, was added to see whether the sum (R+S) of the effects of the rust infection alone (R) and the glume blotch infection alone (S) was different from the effect of both pathogens together in the same plant (I). In all combinations, the difference between two concomitant observations was calculated for each of the sampling dates. For each type of response, the regression of the difference on time was calculated using the model $y = a + bx$, where a and b are constants, and y is the expected value of the difference at time x. The t-values of the parameters in the regression equations were calculated, and subjected to Student's t-test for significance at the 0.10 level. A significant difference from zero of one of the parameter estimates a and b was accepted as evidence that the effects of two disease treatment levels were systematically different.

The second step was the calculation of points of regression curves for each of the responses and for each of the levels of disease treatment. The choice of the models used in the calculations depended on the expected shape of the curves. For total dry weight per plant and total transpiration over the full growth period a sigmoid curve was expected, and a logistic regression was applied (Nair, 1954): $y = k/(1 + be^{cx})$, where b, c and k are constants, y is the expected value of the response at time x, and e the base to the natural logarithms. For the period from inoculation to harvest, only

segments of sigmoid curves could be expected, and here a three parameter semi-logarithmic regression model was applied: $y = a + be^{cx}$, where a, b and c are again constants. For technical reasons, a two-parameter semi-logarithmic regression model was used in cases where a decrease in the response value y with time could not be excluded: $y = be^{cx}$.

Materials and techniques

The description of the environment follows the guidelines proposed by the A.S.H.S. Committee on Growth Chamber Environments (1972).

The environment

The climate chamber. The main voltage was 220 V, 50 Hz. The climate chamber was c. 4 m long, 2.4 m wide, and 2.5 m high (Fig. 1 and Fig. 2).

Light sources. Three types of lamp were used simultaneously; three rows of five 400-W Philips HPLR in a grid arrangement, spaced 0.5 m between centres; two rows of 40-W incandescent lamps suspended between the rows of HPLR bulbs, spaced at 0.2 m intervals; two rows of 40 W 33 RS Philips TLMF fluorescent tubular lamps, placed almost vertically near the long walls of the chamber, 0.06 m apart. Differences in light intensity of less than 20% between the HPLR lamps were attributed to differences in the ages of the lamps. Distances between lamps, and from lamps to plant tips were selected so that the radiation cones of the lamps overlapped in the 'experimental area' at the level of the plant tips. Heat shields between the plants and the lamps were not used to avoid interruption of the vertical air flow in the chamber, reduction of the heat dissipation from the lamps, and interception of the near-UV radiation (360–380 nm). The latter is of critical importance in the formation of pycnidia (Van der Wal et al., unpublished).

Light intensity. Light intensity was measured with two instruments. One was a spherical light intensity meter (photocell, measuring angle $4\pi r$, cosine corrected; Wassink and Van der Scheer, 1951), calibrated in lux and in $W.m^{-2}$ for HPLR lamps. During the experiment, the light intensity at various points between the plants was measured eight times. At plant tip level, 0.75 m under the HPLR lamps, the intensity was 45 Klux, equivalent to 350 $W.m^{-2}$. The platforms supporting the plants were lowered when the distance between plants and HPLR lamps became less than 0.75 m. When the plants reached their maximum leaf area, the light intensity between the plants at 0.10 m above soil level was c. 3 Klux (c. 23 $W.m^{-2}$). The other instrument was a KIPP solarimeter (thermopile, measuring angle $2\pi r$, without cosine correction, type CM 5). At plant tip level c. 180 $W.m^{-2}$ was recorded, confirming the result obtained with the spherical light meter; the latter records values roughly twice those recorded by flat light meters in comparable situations.

Photoperiod. A diurnal cycle of 16 h light and 8 h dark was employed. The fluorescent and incandescent lamps, and four of the HPLR lamps were switched on at the beginning of the light period. Then, at approximately 10 minutes' intervals, the remaining

Table 1. Diurnal pattern of temperature and relative humidity of the air entering the climate chamber, as registered by means of the sensors of the climate chamber control equipment (CS in Fig. 2). The calculated daily mean temperature was 17.2 °C. Note the difference between the daily mean temperature of the incoming air and the mean temperatures in the experimental area between the plants (Table 2).

Period (h) from—to	Temperature (°C) from—to	Rel. humidity (%) from—to
07.15–11.15	14.8–20.1	80–76
11.15–19.00	20.1–20.0	76–78
19.00–23.05	20.0–14.7	78–75
23.05–07.15	14.7–14.8	75–80

Tabel 1. De dagelijkse gang van temperatuur en relatieve luchtvochtigheid van de in de klimaatkamer binnenstromende lucht, geregistreerd met behulp van de meetvoelers van de regelapparatuur (CS in Fig. 2). De berekende gemiddelde dagtemperatuur is 17.2 °C. Zie het verschil tussen de gemiddelde temperaturen van de instromende lucht en die van de lucht tussen de planten in het proefvak (Tabel 2).

HPLR lamps were switched on in two groups of four and a final group of three. At the end of the light period, the lights were switched off over a half-hour period in the same order.

Air movement. The general pattern of air movement is indicated in Fig. 1. Incoming air at c. 0.1 m from the wall had a velocity of c. 0.6 $m.s^{-1}$, measured with a Hastings non-directional wind velocity probe (type N-7B; meter model ERM-1, Hastings). At flag leaf level between the plants the wind speed was c. 0.3 $m.s^{-1}$. Although there was a regular fluctuation in windspeed with a period of a few minutes, no systematic changes in average windspeed occurred during the experiment. The measurements were repeated eight times at fortnightly intervals.

Air temperature. Table 1 shows the temperature pattern of the incoming air recorded by the climate chamber control equipment throughout the experiment. The temperature in the growth chamber differed considerably from that of the incoming air. A distinct temperature profile in the 'experimental area' was caused by the large heat output of the lamps in the absence of a heat shield. Air temperatures between the plants were measured with 0.5 mm shielded copper constantan thermocouples. Soil temperatures were measured with similar thermocouples mounted in the tips of 0.15 m injection needles (Schurer, pers. commun.). Standard aluminium laboratory stands were used to support thermocouples at 0.10 m (lower leaf level), 0.40 m (second leaf level), and 0.70 m (flag leaf level) above the soil surface. Towards the end of the experiment sensors were added at 1.00 m (head level) above the soil surface. Four stands were arranged so that two were in the centre of the inner experimental area and two near the border (Fig. 1 and 2, Table 2).

Leaf temperature. Leaf temperature was measured with a 'Stoutjesdijk' infrared radiometer (Stoutjesdijk, 1966). Generally, leaf temperature was a few tenths of a degree C lower than that of the ambient air at the same time and position in the climate chamber. Temperature measurements of various plant organs indicated that

Table 2. Maximum (T_{max}), minimum (T_{min}), and mean (T_{mean}) temperatures measured in the experimental area between the plants by the sensors on the four masts (Fig. 1 and 2). The coefficients of variation (CV) of the measurements indicate the temperature variations in the horizontal plane. The figures are derived from the full-hour records during one day, with the buckets at level 2 (Fig. 1).

Height above soil (m)	T_{max}(°C)			T_{min}(°C)			T_{mean}(°C)	
	value	time	CV	value	time	CV	value	CV
1.00	25.0	18.00	0.07	14.6	06.00	0.02	20.1	0.04
0.70	24.5	18.00	0.02	15.0	06.00	0.01	20.0	0.02
0.40	23.6	18.00	0.04	14.7	06.00	0.03	19.4	0.03
0.10	22.9	18.00	0.02	15.2	06.00	0.02	19.4	0.01
-0.05	21.5	20.00	0.02	17.4	09.00	0.04	19.6	0.004
-0.15	21.3	20.00	0.01	16.9	09.00	0.04	19.3	0.004

Tabel 2. Waarden en tijdstippen van de maximum (T_{max}), minimum (T_{min}) en gemiddelde (T_{mean}) temperaturen gemeten in het proefvak tussen de planten door meetvoelers aan de vier masten (Fig. 1 en 2). De variatiecoëfficienten (CV) van de metingen met behulp van de vier meetvoelers op de aangegeven hoogten worden gegeven om de temperatuurvariaties in het horizontale vlak aan te duiden. De gegevens zijn ontleend aan metingen op de hele uren gedurende één dag, met emmers op niveau 2 (Fig. 1).

there were temperature gradients along the plants related to the temperature gradients of the ambient air.

Air humidity. The humidity changes of the incoming air, recorded by the growth chamber control equipment, are shown in Table 1. Shielded wet and dry bulb unventilated thermocouples (Schurer, 1972) were used for the measurement of humidity among the plants. The thermocouple elements were calibrated in a radiation-free chamber against high-precision thermometers at a wind speed (c. 0.4 $m.s^{-1}$) similar to the wind speed recorded around the plants in the climate chamber. The humidity sensors were placed at 0.10 m and 0.40 m above the soil surface (Fig. 1) at the 0.10 m level, relative humidity was in the range of 70 % to 80 %, and at the 0.40 m level it was in the range of 60 % to 75 %.

CO_2. The air supply for the climate chamber was drawn from about 20 m above ground level and was filtered to remove dust and other particles. The rate of circulation through the climate chamber was c. 6,000 $m^3.h^{-1}$. The leakage from the chamber because of internal overpressure was c. 150 $m^3.h^{-1}$. The average growth rate being c. 0.3×10^{-3} kg. $plant^{-1}$. day^{-1} dry matter for the 200 plants in the chamber, the rate of CO_2 fixation was c. 2 $moles.day^{-1}$ or c. 0.05 $m^3.day^{-1}$ (0.002 $m^3.h^{-1}$). Assuming that the CO_2 concentration of the incoming air was 0.03 %, the CO_2 requirement of the plants was c. 5 % of the CO_2 input to the climate chamber. The CO_2 consumption was partially compensated by the CO_2 exhaled by persons working in the climate chamber.

Root medium. A commercial potting soil, TRIO 17, was used with an addition of some clay. An analysis of the mixture is given in Table 3. About 1.2 kg of soil (dry weight 0.65 kg) was transferred to polythene bags, 0.20 m long and 0.25 m deep, which were packed to form a flat-sided unit about 0.04 m wide. The lower ends of the

Table 3. Results of an analysis of the soil, made at the beginning of the experiment by the Laboratory for Soil and Crop Testing.

Ph– water:	5.9
Organic matter:	44 %
NaCl:	330 ppm
N– water:	1540 ppm
P– water:	440 ppm
K– water:	1260 ppm
MgO–NaCl:	2138 ppm

Tabel 3. Resultaten van een grondanalyse, uitgevoerd bij het begin van de proef door het Bedrijfslaboratorium voor Grond- en Gewasonderzoek.

bags were perforated. Six such bags were placed side to side in rectangular polythene buckets, 0.27 × 0.20 m at the mouth, and 0.27 m deep. Seeds of wheat cv. Kolibri were pregerminated in petri dishes for 2 days. One seed per bag was planted centrally c. 0.025 m below the soil surface, so that each bucket contained a row of 6 plants at c. 0.04 m distance within the row. This arrangement made possible the removal of a single plant from the climate chamber with a minimum of disturbance to the remaining plants. The bags did not fill the entire volume of a bucket: the gaps remaining between the bags and the walls of the bucket facilitated watering and aeration of the soil.

Water. The maximum water content of the soil during the experiment was 1.5 kg water per 2.5 kg soil, giving a water potential of c. -80 $J.kg^{-1}$. Tap water was added on alternate days from sowing to stage 30, and thence daily until harvest. A bucket received not more than one litre at a time. The result was a fluctuation in the water content of the soil and a variation in soil water potential approximately within the range of -80 $J.kg^{-1}$ to -370 $J.kg^{-1}$. The latter value was the lowest encountered in the samples taken for determining the water potential. The mean value of all samples taken immediately before watering was -212 $\pm$ 19 (S.E.) $J.kg^{-1}$, measured by thermocouple psychrometry. Water was added between the bags and the walls of the buckets, and some water was also applied to the open soil surface to ensure that the fertilizer was washed into the soil.

Nutrients. Nineteen days after sowing, 10^{-3} kg pulverized commercial fertilizer (Granumix 18%N, 18% P, 12% K) per plant was applied to the soil surface at least 0.05 m from the base of the stem. No visible trace remained there after one week.

The host

Seed. The seeds of 'Kolibri' used in this experiment were harvested from previous experiments in the climate chamber, and were free of common pathogens such as *Fusarium* spp. and *Septoria* spp. They had been stored in the coldroom at 4 °C. Germination percentage was over 95. Selected seedlings of equal size were planted in polythene bags as described under *Root medium.*

Dry weight, leaf area, development. After removal of the sample plants from the growth chamber, the soil was washed off the roots, the leaf area was measured by a photo-electric method (ref. Automatic type AAM-5 leaf area meter, Hayashi Denko Co. Ltd, Japan), and the axial development was determined. Leaves per axis and leaf area per axis were recorded, and the leaves, stems (with sheaths), and heads of each axis were grouped together and dried overnight in an oven at 105 °C. A comprehensive analysis of changes in axial distribution and weight of plant organs in the course of time was, however, beyond the scope of this experiment.

The rust

Prevention of cross infection. By proper setting of the climate chamber, using a separate incubator for the inoculations, and inoculating with pathogens that require free water on the leaf surface for spore germination and penetration, it was possible to place uninfected control plants next to abundantly sporulating rusted plants and plants heavily infected with glume blotch. The application of fungicides in order to keep the control plants free of infection was therefore not needed.

Rust inoculum. The rust material used was a mixture which originated from the Flamingo race, isolated by Zadoks in 1961 as No. 1037. Uredospores in quantities of the order of 10^{-2} kg were produced before the commencement of this experiment. The spores were stored in glass ampoules under liquid nitrogen (Loegering et al., 1961).

Timing of inoculation. The rust inoculation was performed at the 75 % heading stage, scale value 56 (Zadoks et al., 1974), 60 days after planting. At this growth stage about half of the final dry matter production has accumulated. Rooting and tillering have been completed, leaf area is at its maximum. The effect of the disease treatment at this stage must be sought mainly in the grain filling.

Inoculum density. An inoculum density of c. 20 spores.mm^{-2} leaf surface was required to achieve a 10 % infection at the end of the latent period. The relation between inoculum density and % of infection of this pathogen-cultivar combination had been established previously. A 10 % infection is serious when it occurs in the field at this stage of development, but usually not disastrous. Increases in the percentage of infection were a result of growth of the pathogen, and were not caused by re-infection. Each bucket of plants was inoculated separately in a settling tower (Eyal et al., 1968), diameter 0.50 m, height 2.50 m, with 50 mg of uredospores, disposed by means of a CO_2 gun. There was no significant difference in the percentage of infection between plants in different buckets. Spore densities achieved in the settling tower were mea-

Table 4. The development of the uninfected plant (C) with time (t) in days after planting. The development is expressed in the decimal scale of Zadoks et al. (1974).

C	05	11	30	32	50	60	71	85	87	92
t	0	7	28	36	44	51	70	83	92	98

Tabel 4. De ontwikkeling van de niet-geïnfecteerde plant (C) in de loop van de tijd (t) in dagen na het planten. De ontwikkeling is aangeduid met behulp van de decimale schaal van Zadoks et al. (1974).

sured by placing vaseline smeared microscope slides horizontally in the tower near the flag leaves of the plants. Densities of 20 to 30 spores.mm^{-2} were found on the slides, and there were no significant differences between slides. The percentage of germination of the spores on the leaf surface was determined after the incubation period using collodion strips and lactophenol cotton blue; c. 82% spore germination was found.

Incubation. Incubation was carried out in a cabinet 2.00 × 1.25 × 2.20 m, with an inner lining of muslin draped over a metal frame, which fitted inside a large metal tray with water. Above the muslin ceiling, a copper pipe with numerous 0.5 mm perforations was mounted over the full length of the cabinet. By this means water was sprayed from the outside over the muslin curtain. The water was collected in the tray and was allowed to drain away. The entire structure was covered by a black polythene sheet. The mean internal temperature was c. 18 °C. Inoculated plants remained in the incubator overnight for about 11 h, and were then returned to the climate chamber.

Disease assessment. The disease assessment was based on the standard scales compiled by W. C. James (1971). Ten days after inoculation the percentage of infection was between 10 and 25.

The glume blotch

Inoculum. After flowering, plants grown in the growth chamber were inoculated with a mixture of *Septoria nodorum* isolates. Infected seeds were stored in the cold room at 4 °C for prolonged periods. The seeds apparently carried a pure *S. nodorum* infection. After mild surface sterilization with hypochlorite followed by washing in sterile water, the seeds were placed on 2% wheatmeal-agar to obtain the fungus in the form of agar plate colonies. When, with a diurnal cycle, these colonies were exposed at room temperature to light containing wavelengths in the range of 360–380 nm, concentric rings of pycnidia were produced within five days. Recently harvested seeds were used as the source of the pathogen, and the agar colonies were checked for irregularities in growth rate, sector formation, and size and density of the pycnidia. Spore suspensions were prepared from the regularly growing colonies only, and after the spore concentration was adjusted to c. 10^5 spores.ml^{-1}, 9 cm ⌀ petri dishes containing wheat meal agar were inoculated with 1 ml of the spore suspension, and incubated at 20 °C under TLMF 40 W 33 RS fluorescent lamps at c. 20 Klux, with a diurnal cycle of 16 h light and 8 h darkness. A yield of about 10^8 spores.dish^{-1} was harvested after 10 days.

Timing of inoculation. The inoculation with *Septoria* was performed after the inoculation with rust but before the rust started to sporulate, so as to avoid re-inoculation by rust in the I treatment.

Inoculum density. The volume and concentration of the spore suspension was adjusted to produce an average spore density of c. 20 spores.mm^{-2} leaf surface in order to obtain a 10% infection. The relation between spore density, incubation and percentage of attack had been established before. A De Villbiss atomizer was employed to spray

the spore suspension on the plants. Spore density was found to be between 20 and 30 spores.mm^{-2}. Spore germination was over 90%.

Incubation. The inoculated plants were incubated overnight for 11 h, as described above, on three consecutive nights, and the plants were kept in the climate chamber during the intervening daytime periods. There was no significant difference in percentage of infection between plants in different buckets on the 8th day after inoculation, when the percentage of infection was between 10 and 25 on the leaves, and c. 15 on the heads.

Management of the experiment

The attitude of the persons taking care of the experiment described here was similar to that of people working in the intensive care unit of a hospital. The instrumentation had attained a high degree of sophistication. The behaviour of the diseased plants was constantly supervised, and at times instant action was required. In order to carry out this aspect of the work it was necessary that at least one person was available at any time during a period of several months.

Transpiration was monitored continuously. Irregularities in transpiration were interpreted as an indication of overwatering, underwatering, or disturbance in climate chamber control. Great alertness was necessary to avoid contamination with common pests such as powdery mildew and aphids. Mildew is difficult to control in climate chambers once it has been introduced. The control of aphids, although possible by periodical treatments with non-persistent organic phosphorous compounds such as DDVP, inhibits the rust and/or injures the rusted plant. The preventive measures were successful and this experiment was carried out without contamination, although both authors were involved in field work during the period of the experiment.

Results

General remarks

The presentation of results reflects two steps in data processing. The first step was a test of differences between concomitant values of a response for all possible combinations of two levels of disease treatment. The regression of differences between the values paired per sampling date on time was calculated; the t-values of the parameters of the regression equations are shown in Table 5. This table allows of comparing the 'noise' in the data with the magnitude of the effects to be studied. Table 6 shows the decisions about the significance of the differences, based on Student's t-test.

The second step was the calculation of points of regression curves for each response. Figures 4, 5, 6, 7, 8 and 9 show the resulting regression curves and Tables 7, 8, 9, 10 and 11 the calculated values of the corresponding parameters.

The climate chamber was set at a diurnal rhythm approximating good weather conditions in the Netherlands at flowering time of spring wheat: a day length of c. 16 h, a day temperature of c. 25 °C and a night temperature of c. 15 °C. With such an environment during the whole growth period, rapid growth and development in the earlier stages of the plant could be expected. The growth period was c. 3.5 months, which is c. 1.5 months shorter than the growth period of spring wheat in the field.

Table 5. The t-values of the regression constants (a) and the regression coefficients (b) are given for seven combinations of treatment levels and six responses. These values were obtained after regression of the differences between two concomitant values (y_i) per observation day (x_i) versus time (x). Details see text. There are n-2 = 4 degrees of freedom for these t-tests, except for kernel weight and kernel number where n-2 = 2. C-R, C-S, and C-I were tested one-tailed, the others two-tailed ($p \leqslant 0.10$). C = uninfected plant; R = plant infected with rust; S = plant infected with Septoria; I = plant infected with rust and Septoria.

	Roots		Leaves		Stems		Heads		Kernel weight		Kernel number	
	a	b	a	b	a	b	a	b	a	b	a	b
C–R	0.96	–1.08	0.91	–0.97	0.99	0.71	0.07	1.30	–1.07	1.49	–0.49	1.83
C–S	–0.28	0.61	–1.01	2.33	–1.98	2.96	–2.29	4.83	–1.12	1.45	–0.41	1.80
C–I	1.25	0.13	0.31	0.20	0.43	1.03	0.67	3.28	3.22	–0.10	8.48	–4.71
R–S	–1.28	1.77	–2.00	3.35	–5.30	4.45	–2.69	3.56	2.66	–1.47	–0.33	1.53
R–I	0.21	1.74	–0.85	1.55	–0.39	0.55	0.95	3.24	3.04	–1.71	6.05	–3.88
S–I	2.46	–1.02	1.04	–1.65	2.28	–1.72	7.91	–1.11	3.17	–1.79	3.39	–2.89
C–R–S+I	–0.40	–0.42	–0.18	0.61	–1.14	1.73	4.45	0.41	–1.93	1.70	–3.05	2.82

Tabel 5. De t-waarden van de regressieconstanten (a) en de regressiecoëfficienten (b) zijn vermeld voor zeven combinaties van behandelingsniveaus en voor 6 responsies. Deze waarden zijn verkregen na regressie van het verschil van twee bijeengevoegde waarden (y_i) per waarnemingsdag (x_i) tegen de tijd (x). Zie de tekst voor details. Er zijn n–2 = 4 vrijheidsgraden voor de t-toets, behalve by 'kernel weight' en 'kernel number', waar n–2 = 2. C–R, C–S en C–I zijn eenzijdig getoetst, de overige combinaties tweezijdig ($p \leqslant 0.10$).
C = niet geïnfecteerde plant; R = plant geïnfecteerd met roest; S = plant geïnfecteerd met Septoria; I = plant geïnfecteerd met roest en Septoria.

Table 6. The decisions about the significance of the differences between disease treatment levels, listed per disease treatment combination (C–R,... C–R–S+I) and per response. A + (plus) indicates significance, a – (minus) no significance at a confidence limit $p \geqslant 0.90$. The decisions are based on Student's t-values and the degrees of freedom given in Table 5.

	Roots		Leaves		Stems		Heads		Kernel weight		Kernel number	
	a	b	a	b	a	b	a	b	a	b	a	b
C–R	–	–	–	–	–	–	–	–	–	–	–	–
C–S	–	–	–	+	+	+	+	+	–	–	–	–
C–I	–	–	–	–	–	–	–	+	+	–	+	+
R–S	–	–	–	+	+	+	+	+	–	–	–	–
R–I	–	–	–	–	–	–	–	+	+	–	+	+
S–I	+	–	–	–	+	–	+	–	+	–	+	–
C–R–S+I	–	–	–	–	–	–	+	–	–	–	+	–

Tabel 6. Beslissingen over de significantie van de verschillen tussen ziektebehandelingsniveaus, gerangschikt per ziektebehandelingscombinatie (C–R,... C–R–S+I) en per responsie. Een + betekent significantie, een – geen significantie bij een betrouwbaarheidsgrens van $p \geqslant 0.90$. De beslissingen berusten op de t-waarden van Student en de vrijheidsgraden vermeld in Tabel 5.

Table 7. Parameter values estimated by means of the calculation of the regression of dry matter data and kernel number on time. The regression equations used are $y = a + be^{cx}$ for the responses leaves, stems, heads and kernel weight, and $y = be^{cx}$ for roots and kernel number. Dimensions as in Fig. 4. – (minus) means that no value has been estimated, *means that a non-relevant estimate has been made, because of certain properties of the computer programme used. It indicates that the variance in data as compared with the increase with time was too large for a proper estimate to be calculated. The regression curves are shown in Fig. 4.

		Roots	Leaves	Stems	Heads	Kernel weight	Kernel number
C	a	–	3.8	8.4	11.3	7.3	–
	b	7.2	*	*	−1217	*	319
	c	− 0.015	*	*	− 0.071	*	−0.004
R	a	–	3.7	6.9	11.0	6.2	–
	b	2.8	*	*	−150	*	370
	c	− 0.004	*	*	− 0.042	*	−0.006
S	a	–	3.3	7.6	7.6	4.7	–
	b	10.3	*	*	−213	*	562
	c	− 0.020	*	*	− 0.057	*	−0.012
I	a	–	3.5	6.9	6.6	4.4	–
	b	8.6	*	*	−87.4	−2809	72
	c	− 0.021	*	*	− 0.042	−0.078	+0.008

*Tabel 7. Parameterwaarden geschat door middel van de berekening van de regressie van droge stof gegevens en aantal korrels op de tijd. De gebruikte regressievergelijkingen zijn $y = a + be^{cx}$ voor de responsies 'leaves', 'stems', 'heads' en 'kernel weights' en $y = be^{cx}$ voor 'roots' en 'kernel number'. De dimensies zijn als in Fig. 4. Een – (minus) betekent dat geen waarde is geschat, een * betekent dat een niet relevante schatting is gemaakt, als gevolg van bepaalde eigenschappen van het gebruikte computerprogramma. Het betekent hier, dat de spreiding in de gegevens in verhouding tot de toeneming in de tijd te groot was voor een goede schatting. De regressiecurven zijn te zien in Fig. 4.*

Fig. 3. Diagram of the axial development of the mature plant. The numbers indicate the order of the axes, modified after Schoute (1910). 0 = the main stem; 1, 2, 3, and 4 = first order axes; ---- = axes not always present; ● = axis usually fertile.

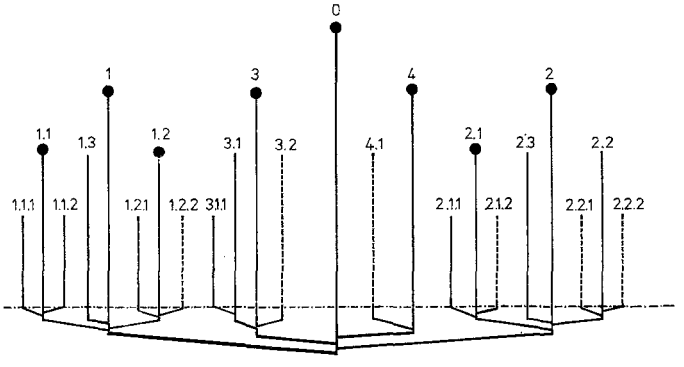

Fig. 3. Diagram van de axiale ontwikkeling van de volwassen plant. De getallen geven de orde van de assen aan volgens het, hier enigszins gewijzigde, systeem van Schoute (1910). 0 = de hoofdhalm; 1, 2, 3 en 4 = eerste orde zijassen; ---- = as niet altijd aanwezig; ● = as gewoonlijk fertiel.

Fig. 4. Mean dry weight (w) per plant organ [10^{-3}.kg.plant^{-1}] and kernel number per plant in C, R, S, and I versus time (t) [day] in the period after inoculation. Symbols as in Table 5: ●— = C, ○— = R, △--- = S, ▲--- = I. For the parameters of the regression curves see Table 7.

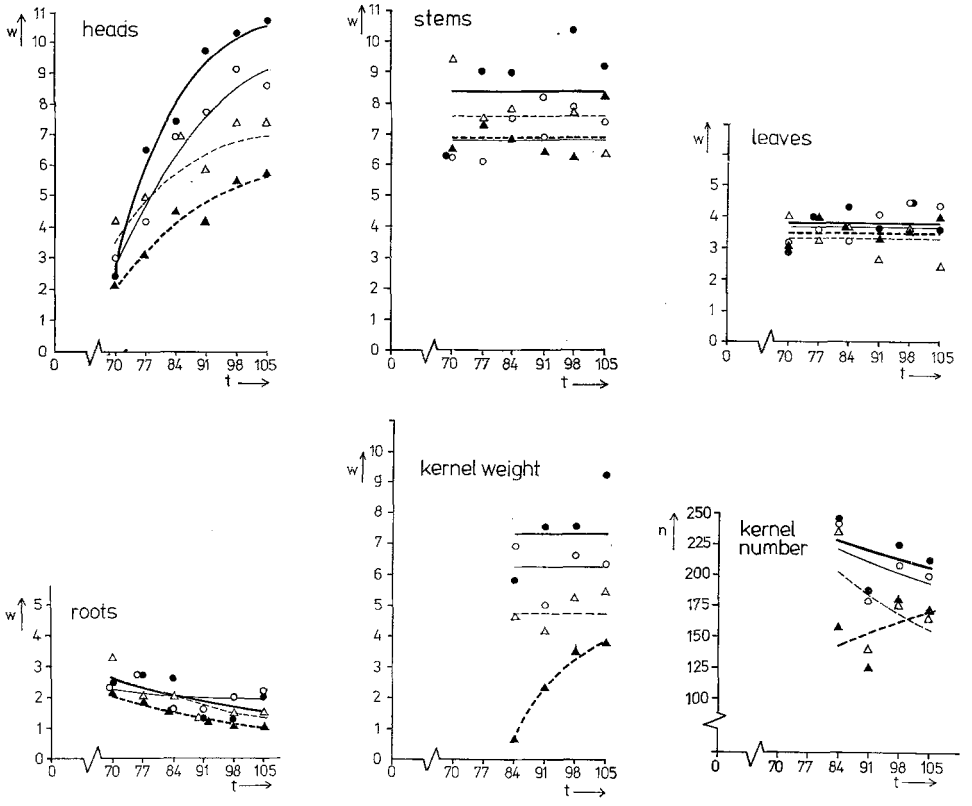

Fig. 4. Gemiddeld drooggewicht (w) per plant-orgaan [10^{-3}.kg. plant^{-1}] en aantal korrels per plant in C, R, S en I tegen de tijd (t) [dag] in de periode na de inoculatie. Gebruik van de symbolen is aangegeven in Tabel 5: ●— = C, ○— = R, △--- = S, ▲--- = I. Voor de parameters van de regressiecurven zie Tabel 7.

Effects of disease treatment on the host

Development. As the inoculation was timed at stage 56, no effects of disease treatment on development from planting to flowering could be expected. At flowering stage, axial development was completed (Fig. 3). Maturation of the diseased plants proceeded faster than in the uninfected plants.

Growth. As expected, the effects of disease treatment became visible mainly in the growth of the heads. The head weights in S and I were lower than in C (Table 5 and 6, Fig. 4). The head weight in I was less than that in S, which in its turn was less than in R. The difference in head weight between C and R was not significant. The loss of head weight in I was larger than the sum of the reductions in S and R, indicating interaction (Van der Wal et al., 1970).

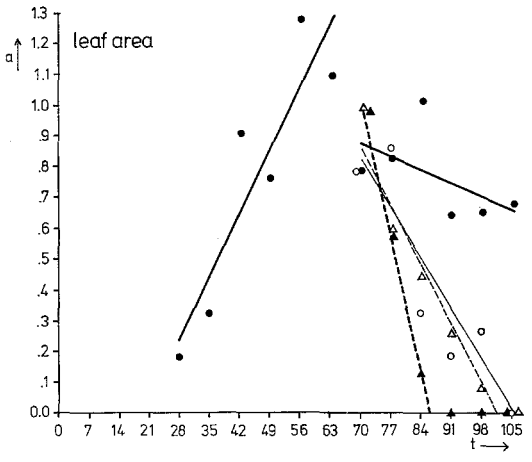

Fig. 5. Mean leaf area [10^{-1}.m^2.plant^{-1}] versus time [day] in C, R, S, and I. For dots and lines see Fig. 4. Parameters of the regression curves are given in Table 8.

Fig. 5. Gemiddeld bladoppervlak [10^{-1}.m^2.plant^{-1}] tegen de tijd [dag] bij C, R, S en I. Voor de punten en lijnen zie Fig. 4. De parameters van de regressiecurven staan in Tabel 8.

Table 8. Parameter values estimated by calculating the regression of leaf area [10^{-4}.m^2.plant^{-1}] on time [day], using the regression equation $y = a + bx$. The periods covered are from day 28 to day 63 for C_1 and from day 70 to day 105 for the treatment levels C_2, R, S, and I. For the explanation of the symbols see Table 5. For the regression curves see Fig. 5.

	a	b
C_1	− 580	+29.2
C_2	1281	− 5.9
R	2429	−23.0
S	2697	−26.2
I	5215	−60.5

Tabel 8. Parameterwaarden geschat door middel van de berekening van de regressie van het bladoppervlak [10^{-4}.m^2.plant^{-1}] op de tijd [dag], met behulp van de regressievergelijking $y = a + bx$. De perioden waarover geschat is lopen van dag 28 tot dag 63 bij C_1 en van dag 70 tot dag 105 bij de behandelingsniveaux C_2, R, S en I. Voor de symbolen zie Tabel 5. Zie ook de regressiecurven in Fig. 5.

The reaction of the leaf area to disease treatment is given in Fig. 5 and Table 8. Only turgescent leaves were measured. The leaf area measured includes the infected areas of turgescent leaves, and excludes leaves killed by pathogens or dead of senescence. The rapid decline of leaf area in I is conspicuous. The leaf areas in R and S differed but slightly. The stems in S and I were c. 0.15 m shorter than those in R and C; the stem weights were correspondingly lower. Root deterioration was rapid in I.

Transpiration. After inoculation, the transpiration in R was higher than in C, but the transpiration in S was less than in C (Fig. 6a and 6b). The transpiration rate in I initially equalled that in R, but later it decreased rapidly. In the end, transpiration in I was lower than in S.

Rust in R and I. The first open pustules were seen on the leaves in I 4.5 days after inoculation, one day earlier than in R. Telia on the sheaths were found after c. 32 days in I and R. The first open uredosorus on the stem between flag leaf and head was

Fig. 6. Mean transpiration [kg.plant^{-1}] in C, R, S, and I versus time [day]: a – in the period from inoculation to harvest; b – enlarged detail from day 68 to day 78. For dots and lines see Fig. 4. Parameters of the regression curves are given in Table 9.

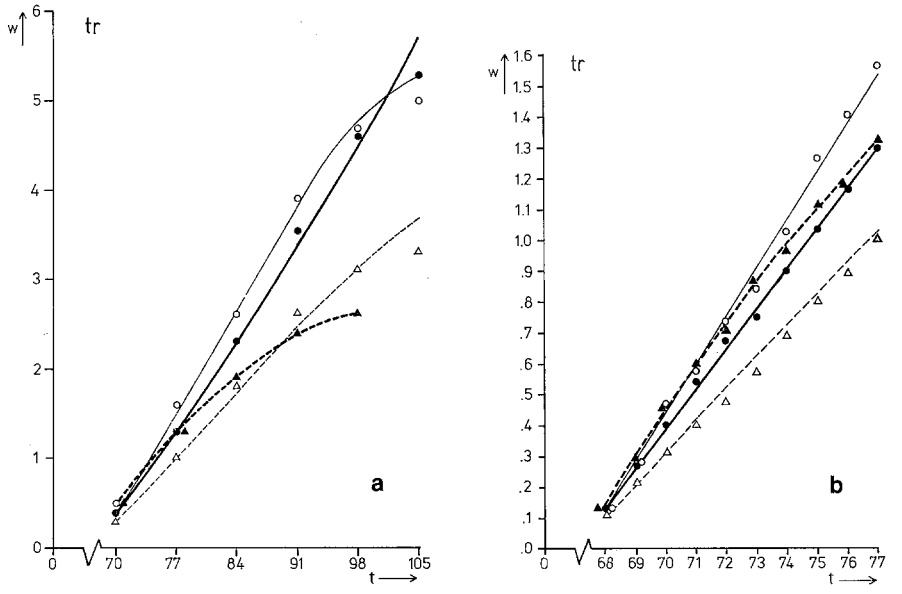

Fig. 6. Gemiddelde transpiratie [kg.plant^{-1}] bij C, R, S en I tegen de tijd [dag]: a – in de periode vanaf de inoculatie tot de oogst; b – detail van dag 68 tot dag 78. Voor de punten en lijnen zie Fig. 4. De parameters van de regressiecurven staan in Tabel 9.

Table 9. Parameter values estimated by calculating the regression of transpiration [kg. plant^{-1}] on time [day], using the regression equation $y = a + be^{c(x-67)}$. For explanation of the symbols see Table 5. For regression curves see Fig. 6.

	a	b	c
C	−16.08	16.09	0.008
R	76.20	−76.23	−0.002
S	16.48	−16.48	−0.007
I	3.15	− 3.19	−0.055

Tabel 9. Parameterwaarden geschat door middel van de berekening van de regressie van de transpiratie [kg.plant^{-1}] op de tijd [dag], met behulp van de regressievergelijking $y = a + be^{c(x-67)}$. Voor de symbolen zie Tabel 5. Zie ook de regressiecurven in Fig. 6.

found 39 days after inoculation, when yellowing of the heads began. The percentage of infection by the rust in R and I is shown in Fig. 7. The increase in infection was not due to re-infection but to secondary pustules. In the linear phase of the curve, the difference in the rate of increase between R and I is obvious, the rate of increase in I being lower than in R. In I, brown rings were observed round the rust pustules, six days after inoculation with glume blotch. Sporulation of the rust came to a stop within a week. Telia of the rust appeared one week earlier than in R. On day 68 the plants were dead. No rust appeared on the heads in either treatment.

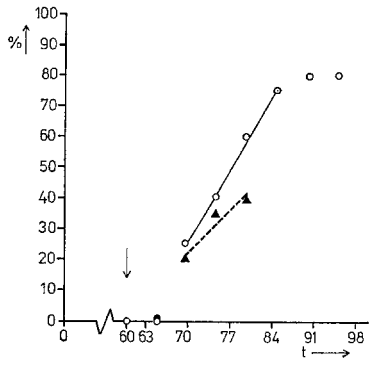

Fig. 7. Mean percentage of infection by rust versus time [day] in R and I. ○— = R, ▲--- = I, ↓ = time of inoculation. For the parameters of the regression lines see Table 10.

Fig. 7. Gemiddeld aantastingspercentage van de roest tegen de tijd [dag] bij R en I. ○— = R, ▲--- = I, ↓ = inoculatietijdstip. De parameters van de regressielijnen staan in Tabel 10.

Table 10. Parameter values estimated by calculating the regression of the percentage of infection on time in the nearly linear part of the curves, using the regression equation $y = a + bx$. See also the regression lines in Fig. 7 and 8.
R_R = rust in disease treatment level R; R_I = rust in disease treatment level I; S_{SI} glume blotch in disease treatment level S on the leaves; S_{Sh} = glume blotch in disease treatment level S on the heads; S_{II} = glume botch in disease treatment level I on the leaves.

	a	b
R_R	−214	3.4
R_I	−118	2.0
S_{SI}	−265	3.9
S_{Sh}	−282	4.1
S_{II}	−458	7.0

Tabel 10. Parameterwaarden geschat door de berekening van de regressie van het aantastingspercentage op de tijd in het bijna lineaire deel van de curven, met behulp van de regressievergelijking $y = a + bx$. Zie ook de regressielijnen in Fig. 7 en 8. R_R = roest in ziektebehandelingsniveau R; R_I = roest in ziektebehandelingsniveau I; S_{SI} = kafjesbruin in ziektebehandelingsniveau S op het blad; S_{Sh} = kafjesbruin op de aren in S; S_{II} = kafjesbruin op het blad in I.

Glume blotch in S and I. Flecks caused by glume blotch were first observed three days after inoculation in I, two days later they appeared in S. The flag leaves showed small dark brown spots, the lower leaves large greyish brown flecks. In S, the first pycnidia were found in the leaves 21 days after inoculation, and two days later also on the heads. At harvest time the nodes in I were sunken, but not in S. The increase in percentage of infection of glume blotch in I was greater than in S (Fig. 8). The heads in S showed a percentage of attack comparable to that of the leaves (Fig. 8). The heads in I discoloured so rapidly that no attempt was made to estimate the percentage of infection. The figures give the impression that glume blotch had been more injurious than rust. The number of spores applied per unit leaf area did not differ appreciably, but the heads were infected by glume blotch and not by rust.

Conclusions

No effect of disease on axial development was found, as could be expected when inoculating at stage 56. Maturation of the plants, especially of those treated with

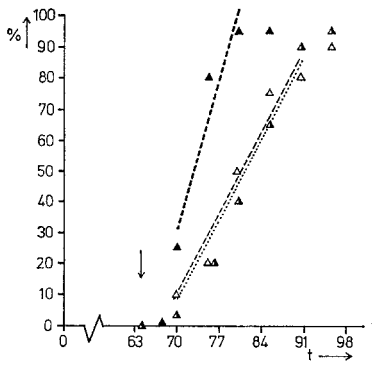

Fig. 8. Mean percentage of infection by glume blotch versus time [day] on leaves (△---) and heads (▲.....) in S, and on leaves (▲---) in I. For the parameters of the regression lines see Table 10.

Fig. 8. Gemiddeld aantastingspercentage ten gevolge van kafjesbruin tegen de tijd [dag] op bladeren (△---) en aren (▲.....) in S, en op bladeren (▲---) in I. De parameters van de regressielijnen staan in Tabel 10.

glume blotch (S and I), was accelerated. The effects on growth were mainly observed in the dry weight of the heads. Loss of dry weight of the heads due to rust (R) was not conspicuous; that caused by glume blotch (S) was significant. The effect of both pathogens together (I) on the dry weight of the heads was greater than the sum of the effects of each of the pathogens separately, so that interaction must have occurred. Kernel weight was lower and kernel number was less in I than in C, R and S.

Reduction of turgescent leaf area after inoculation was fast in I and slow in R and S; the latter two did not differ in this respect.

Transpiration in R was greater than in C, in S smaller than in C. Initially, the transpiration of I was equal to that in R, but later the transpiration rate decreased rapidly to a value far below that in S. Glume blotch infection retarded the growth of rust but accelerated the formation of telia. The percentage of infection by glume blotch increased faster after previous rust infection (I) than without (S), on leaves as well as on heads.

Discussion

The ripening of the uninfected plants in the climate chamber differed from that of a normal crop in the field. At harvest time, the heads were yellow and the kernels were ripe, but the leaves were still green for c. 70 %. In all cases, the kernels were shrivelled, also in C. Logistic regression on growth and transpiration of the uninfected plant over the full growth period showed poor fit in this experiment. The semi-logarithmic regressions fitted much better (Fig. 9a and 9b). The value of the regression parameter c (Table 9) for the transpiration of the uninfected plant in the period after infection was low but positive, indicating that the transpiration rate did not decrease in the course of time.

This effect, known in French as '*échaudage*', may be related to the temperature profile in the climate chamber. The highest air temperature in the climate chamber was measured near the heads, whereas in the field the highest air temperatures during the day are measured c. 0.25 m above soil level in a spring wheat crop. The 'inverted maturation process' (heads first, leaves later) might be described as heat injury.

The conclusions derived from this experiment are summarized in the previous section. The question arises whether they may be generalized, because the magnitude

Fig. 9a. Mean dry weight [10^{-3}.kg.plant^{-1}] versus time [day] in C over the full growth period. For the parameters of the regression curve see Table 11.
Fig. 9b. Mean transpiration [kg.plant^{-1}] versus time [day] in C over the full growth period. For parameters of the regression curve see Table 11.

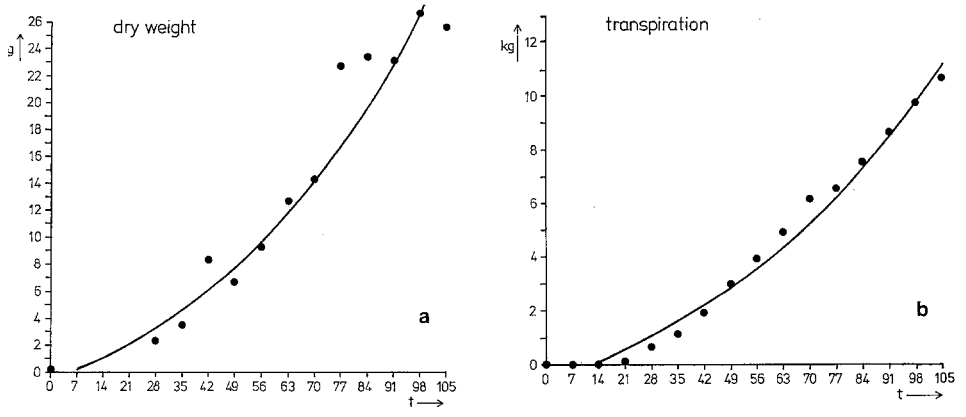

Fig. 9a. Gemiddeld drooggewicht [10^{-3}.kg. plant^{-1}] tegen de tijd [dag] in C over de hele groeiperiode. Zie voor parameters van de regressiecurve Tabel 11.
Fig. 9b. Gemiddeld drooggewicht [kg.plant^{-1}] tegen de tijd [dag] in C over de hele groeiperiode. Zie voor parameters van de regressiecurve Tabel 11.

Table 11. Parameter values estimated by calculating the regression of total dry weight [10^{-3}. kg.plant^{-1}] and transpiration [kg.plant^{-1}] on time [week] in disease treatment level C, using the regression equation $y = a + be^{cx}$. See the regression curve in Fig. 9.

	a	b	c
dry weight	−7.2	6.6	0.117
transpiration	−4.1	3.5	0.098

Table 11. Parameterwaarden geschat door middel van de berekening van de regressie van het totale drooggewicht [10^{-3}.kg.plant^{-1}] en de transpiratie [kg.plant^{-1}] op de tijd [week] bij ziekte-behandelingsniveau C. De gebruikte regressievergelijking heeft de vorm van $y = a + be^{cx}$. Zie ook de regressiecurve in Fig. 9.

of the differences between infected and uninfected plants is as much an outcome of the circumstantial factors as of the experimental factors.

Samenvatting

Een ecofysiologische benadering van 'schade', geïllustreerd aan het systeem tarwe, bruine roest en kafjesbruin.
II. De ontwikkeling, groei en transpiratie van ongeïnfecteerde planten en planten geïnfecteerd met Puccinia recondita f.sp. triticina en/of Septoria nodorum in een klimaatkamerproef.

De proef had tot doel inzicht te verschaffen in het ontstaan van 'schade' door pathogene schimmels bij graangewassen. De infectie verandert in het algemeen de fysiologie van de geïnfecteerde plant. De aard en mate van de verandering hangen vermoedelijk af van de toestand van de plant op het tijdstip van infectie, van de aard en hoeveelheid

van het infectieuse agens, en van de groei-omstandigheden na de infectie. Als objecten werden gekozen de ongeïnfecteerde tarweplant (C), de plant geïnfecteerd met alleen *P. recondita* (R), die met alleen *S. nodorum* (S), en de tarweplant geïnfecteerd met beide pathogenen (I). Voor de beschrijving van de 'fysiologie' van de plant werden gekozen: de ontwikkeling, groei en transpiratie. De axiale ontwikkeling is beschreven volgens Schoute (1910) en het ontwikkelingsstadium volgens Zadoks et al. (1974). De groei is beschreven aan het droge-stofgewicht van de plant als geheel en dat van wortels, spruit, blad, halmen, aren en korrels, terwijl ook het aantal korrels en het bladoppervlak bepaald werden. Deze proef behoort tot de klasse 'single input – multiple output experiments' (Zadoks, 1972). De enige variabele die op meer dan één niveau voorkomt is de variabele 'ziekte-behandeling' met vier niveaus: C, R, S en I). Alle overige variabelen zijn 'situatiefactoren', waarvan een beschrijving gegeven wordt.

De inoculaties werden per pathogeen slechts eenmaal in de groeicyclus van de tarweplant gedaan, terwijl herinfectie werd uitgesloten. De roestinoculatie vond plaats 60 dagen na het planten in ontwikkelingsstadium 56, vier dagen later volgde de inoculatie met *Septoria*. De axiale ontwikkeling van de plant werd niet beïnvloed. Wel volgden de ontwikkelingsstadia elkaar sneller op bij de zieke planten. De groei werd duidelijk beïnvloed. Het verlies aan drooggewicht van de aren was niet opvallend in R, wel in S. Het effect van beide pathogenen samen in I op het verlies van aargewicht was groter dan de som van de effecten van elk van de pathogenen afzonderlijk; dit is een aanwijzing voor het bestaan van interactie. In I was het korrelgewicht lager en het aantal korrels kleiner dan in C, R en S (Fig. 4, Tabel 6).

De vermindering van turgescent bladoppervlak na inoculatie verliep snel in I, minder snel in R en S. De vermindering van het turgescente bladoppervlak in R en S verschilde onderling niet. De transpiratie in R was groter, die in S kleiner dan in C. Aanvankelijk was de transpiratiesnelheid in I gelijk aan die in R, maar later nam de transpiratiesnelheid sterk af. Uiteindelijk was de transpiratie in I veel geringer dan in S (Fig. 6, Tabel 9). De toeneming van het aantastingspercentage door de roest was in I geringer dan in R (Fig. 7). De roestsporulatie in I hield spoedig na het zichtbaar worden van de kafjesbruinsymptomen op. De toeneming van het aantastingspercentage door *Septoria* in I was groter dan die in S, zowel op het blad als op de aar (Fig. 8).

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