EFFECTS OF ENVIRONMENT ON BIOLOGY OF

PUCCINIA HELIANTHI AND ON DEVELOPMENT

OF RUST ON SUNFLOWERS

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I.   INTRODUCTION

Atmospherical environmental factors have long been known to affect rust development on green plants. Environmental conditions subsequent to infection usually have more effect than conditions prior to infection. Moisture, temperature, and light have been shown to be most significant of the atmospheric factors. These factors may affect: a) inoculum efficiency, as appraised by the density of uredial pustules formed, b) length of incubation period, c) reaction type, d) germinability of uredospores produced, and e) formation and infectivity of teliospores. The effects may vary with the race-variety combination of pathogen and host. Knowledge of the significance of the various components of the environment, singly and in combination, on the development of parasitic diseases is extremely important to epidemiologists and to plant breeders attempting to produce disease-resistant varieties of plants.

The studies, reported herein, are of a preliminary nature. They are concerned with sunflower rust (Puccinia helianthi Schw) which possesses the capability of causing severe and damaging epidemics on Helianthus annuus L. They were undertaken to explore the significance of atmospheric factors upon the development of its sporophytic stage beginning with uredospore inoculum. The time available restricted the research to a consideration of two factors, temperature and light, which it was thought might provide

the most useful and interesting data. While the moisture
factor is extremely important, its role in the development
of the sporophytic stages of rust fungi is likely to be
more simple in its action and more readily conjectured
from knowledge of studies on other rust fungi than are the
influences of either temperature or light.

II.   REVIEW OF SELECTED LITERATURE

The literature selected for review is not intended
to be exhaustive but rather to illustrate the different
known aspects of the influence of temperature and light
upon the course of development of foliage rusts and upon
the reaction types induced in infected hosts.

Most investigators have conducted their experiments
under the unnatural condition of constant temperatures
while only a few have probed the effects of fluctuating
temperatures such as the more or less rhythmical day and
night changes which occur during a twenty-four-hour period.
In this review, the temperatures given are constant unless
it is stated that temperature fluctuations are involved.

A.   TEMPERATURE EFFECTS

In general, it appears that temperature affects
pathogens and host responses to them to about the same
degree. The effects of constant temperatures will be
mentioned first followed by those caused by fluctuating
temperatures.

1. Intensity of Infection

Intensity of infection can be judged, though not
always with strict accuracy, from the number of pustules per
unit area of leaf surface following inoculation of plants.

Peltier (1923) placed three varieties of wheat inoculated with Puccinia graminis tritici at 15°, 20°, 25°, and 30°C, with optimum moisture conditions. There was a sharp optimum at 25°C for density of pustules, contrasted to a broad optimum, 10° to 28°C, for spore germination.

The optimum temperature for infection of beans by Uromyces phaseoli is 18° to 21°C, whereas for spore germination and germ tube elongation it is 12° to 18°C (Shands and Schein, 1962).

The significance of temperatures for infectivity is illustrated by the data of Lange et al.(1958) who found that infection of wheat by 7 strains of P. graminis tritici fell off by about 10% for each degree below the optimum of 75 ± 1°F (24°C) and at a slightly faster rate for each degree above the optimum. The minimum for infection was near 60°F (15.6°C) and the maximum was 85°F (29.4°C).

The studies of Sharp et al.(1958) and of Emge (1958) demonstrated that the highest infectivity of uredospores of P. graminis tritici was promoted by temperatures within the range of 60-75°F (15.6-23.9°C) during germination, growth of germ tube and appressorial formation while post-appressorial development was best at 85°F (29.4°C). In nature, during the rust season, temperature is often quite low during a large portion of a dew period and this is followed by higher temperatures after the sun rises. The results of Sharp et al.(1958) indicate a close adaptation of stem rust to take advantage of these diurnal temperature changes.

Preinoculation temperatures may also be important.
Mohamed (1961) observed more infection centers of stem rust
on inoculated wheat seedlings exposed for some days to
85°F (29.4°C) than on those similarly exposed to 75°F
(23.9°C) previous to inoculation.

2. Incubation Period

The length of incubation refers to the time elapsing
from application of inoculum to appearance of symptoms or
sometimes in the case of rusts to the appearance of
uredospores which follows in one to a few days (often depend-
ing upon prevailing temperatures) after the beginning of
symptom expression.

As temperatures are increased above or reduced below
the optimum, the incubation period progressively lengthens
(Dimock and Baker, 1951, Lange et al, 1958, Naoumova, 1935,
Schein, 1961, Stakman and Levine, 1919, Stakman and Harrar,
1957, and Zimmer and Schafer, 1961). Stakman and Levine
(1919) found that stem rust of wheat developed best between
66.5° and 70°F (19.1° and 21.1°C). They observed a
retardation in the time of fruiting of 1 day for every rise
in temperature of 10 degrees above 70°F (21.1°C) and of 1
day for every fall of 5 degrees below 66.5°F (19.1°C). At
this temperature (19.1° - 21.1°C) most flecks appeared in
from 5 to 7 days and uredinia developed within another day
or two.

Depending on temperature, the time required for commencement of uredospore production by the wheat stem rust fungus on a susceptible variety can vary from 5 days to 3 months. The incubation period for P. coronata is 5 to 6 days at 25°C; 9 days at 20°C; 13 to 15 days at 15°C; and 15 days at 13°C (Stakman and Harrar, 1957).

Invasion of host tissues by P. triticina and the onset of fruiting are favoured by fluctuations in temperature (cool nights and warm days) as reported by Chester, (1946).

Naoumova, (1935) studied the influence of temperature on the incubation period of P. triticina. She showed that the day temperature fluctuations between 15.6° and 23.6°C and between 18° and 29°C did not appreciably affect the growth of the intramatrical mycelium, but temperatures above 30°C for several hours significantly lengthened the incubation period. Great fluctuations of the day temperature between 35° and 55°F (1.67° and 12.8°C) seemed to stimulate the development of the internal mycelium.

According to Dimock and Baker(1951) P. antirrhini, snapdragon rust develops more vigorously and sporulation is definitely more luxuriant in plants exposed to fluctuating temperatures in the greenhouse than in plants held at constant temperatures. In 1961b, Schein demonstrated that diurnal temperature changes during the colonization and fruiting periods of U. phaseoli can influence symptom development and

sporulation. Symptom expression was more rapid at 80°-70°F (26.7° - 21.1°C) day-night temperatures than at 70°-60°F (21.1°- 15.6°C) day-night temperatures. The coolest environment employed (21.1°-15.6°C) retarded the appearance of symptoms by one day and the attainment of full sporulation by 2 days. A 90°-60°F (32.2°-15.6°C) regime completely inhibited symptom development. The data show the fallacy of using mean temperatures to characterize the influence of temperature upon this rust under artificial or field conditions.

3. Classes and Infection Reaction or Types

The leaf reaction classes and infection types within each class are employed for the detection of different degrees of host resistance and the identification of physiologic races of cereal rust (Stakman, Stewart and Loegering, 1962). Phenotypic variability in the infection types, especially in cereals attacked by rusts has often been reported in the literature. Temperature and light have especially strong ability to alter host resistance, particularly when it is either type 2 or X.

Among the first to give attention to the temperature influence was Peltier (1923) and Peturson (1930), who showed that postinoculation temperature influenced the types of infection produced by physiologic forms of P. coronata avenae on varieties Green Mountain and White Tartar. In 1935, Murphy observed that oat varieties Green Russian, Hawkeye, Anthony, Sunrise, Green Mountain and White Tartar were

resistant to form 7 at 55°F (12.8°C) but susceptible at 85°F
(29.4°C), while at an intermediate temperature a mesothetic
reaction was produced on all these varieties. Other varieties,
such as Red Rustproof, Sterisel, Belar, Glabrota, Victoria,
Bond and Markton did not change from resistant to susceptible,
or vice versa, but exhibited only a small decrease in resist-
ance or a small increase in susceptibility as the temperature
became higher. In 1937, Wei demonstrated that the reaction
type of the susceptible and resistant bean varieties to rust
is very stable and influenced very little, if at all, by
variation in the temperature factor. It was found that at low
temperatures (6.11°-21.1°C) the Kenya variety 318 A.J.4-A-1
of wheat was resistant to 11 races of P. graminis tritici, but
at higher temperatures (18.3°-34.4°C) it became susceptible
to 6 of the races; four other wheat varieties did not show
this varying reaction (Joshi, 1962).

Changes in reaction type are usually quite small. This
is illustrated well by the responses of inbred lines of corn
to specific lines of P. sorghi at different temperatures as
reported by Syamanandra and Dickson (1959). Most of the
temperature-induced changes were from one susceptible type to a
very similar one. A suboptimal temperature of 16°C brought
about a change from the fully susceptible type 4 reaction to
the highly resistant necrotic fleck reaction type 0; when
inbred corn line Pop 35 was inoculated with rust lines 921 and
925. Zimmer and Schafer (1961) reported a shift in reaction

from type 0 at 60°F (15.6°C) to type 3 at 80°F (26.7°C) in the case of P. coronata on an oat variety; Schein (1961a) reported an alteration in reaction of Pinto 111 lima beans to U. phaseoli from the normally susceptible type to a highly resistant necrotic one when inoculated plants were held for 7 days after inoculation at 32°C.

In 1961a, Bromfield studied the effect of temperature on a mixed reaction (mesothetic or X type) on wheat in response to P. graminis tritici which was characteristically produced on a temperature sensitive variety of wheat subjected to a post-inoculation temperature of 72°-74°F (22.2°-23.3°C). At temperatures below these values, the reaction was in the resistant class and at temperatures above, in the susceptible class. Similar findings were reported by Wei (1937) for bean rust.

Precise temperature control has demonstrated that differences of only 2° or 3°F may shift reactions from one class to another. The susceptibility developed at high temperatures or the resistance at low temperatures were not retained when the temperature sensitive varieties were subsequently exposed for a sufficient length of time to a sufficiently low or high temperature (Bromfield, 1961b, Zimmer and Schafer, 1961).

The time required for the temperature to alter the reaction seems to be a few days. Diurnal temperature changes are probably unimportant. When the host is subjected to the week to week changes in temperature which might occur during

its growth period, the macroscopic appearance of the resulting pustules may be strongly influenced by the value of the temperature and the length of exposure at any given time of their development (Bromfield, 1961b). According to Straib, (1939). summer resistance of wheat to P. glumarum develops principally under the influence of rising summer temperatures which may make a variety virtually immune from yellow rust during the main growing part of the summer; the same variety may bear a great deal of pustules of a susceptible type in the cooler spring or autumn.

By altering the temperature at various stages after penetration of stem rust of a temperature sensitive wheat variety, Silverman (1959) was able to show that the temperature effect came into play about when symptom development and spore formation commences. At this time, the effect of high temperature made the host more susceptible.

High temperature during pustule formation promoted a susceptible reaction while low temperature promoted a more resistant reaction of oats to stem rust. Roberts (1963) observed that the variety Rodney was resistant to race 7 and 8 at 75°F (23.9°C) but susceptible at 85°F (29.4°C). The expression of resistance or susceptibility depended upon the stage of rust development at the time of exposure to high or low temperature and the duration of exposure. Plants transferred to 85°F (29.4°C) before flecking were susceptible, those

transferred at flecking were mesothetic, and those transferred afterwards were resistant. The minimum exposure to either temperature was 4 days although fluctuations in temperature were cumulative in effect. Preinoculation exposure to high temperatures did not affect the reaction. The temperature effect was a localised reaction. New sources of resistance to oat stem rust were differentiated by rust reactions at both high and low temperatures.

In 1960, Mohamed demonstrated that the preinoculation temperature to which wheat seedlings are exposed, may also alter their reaction to stem rust. More infection centers were produced and reaction was shifted to a more susceptible type, if the preinoculation temperature was 85°F (29.4°C) than if it was 70°F (21.1°C). The longer the inoculated seedlings of certain wheat varieties were kept at higher temperatures after inoculation and the higher the temperature up to a certain degree of temperature, the larger were the uredia; this was most pronounced with temperature sensitive varieties. Sharp (1962) found that preinoculation temperature was often critical in determining rust reaction types induced by P. striiformis. Several wheat varieties, including Omar and Idaed were susceptible when grown at 15°C prior to inoculation, but resistant when grown at 24°C in the preinoculation period. Other varieties such as Webster and Holzapfels Fruh were susceptible when preconditioned at 24°C and resistant when

preconditioned at 15°C. Many varieties exhibited identical rust reaction types after being grown at 15° or 24°C. In 1965, Sharp reported that the dark-period temperatures during both the preinoculation and postinoculation phases were critical in determining infection type.

Studies on the effects of temperature on expression of infection type are most complete with wheat stem rust. The results of this work may be briefly summarized as follows; (1) the reaction of most varieties of wheat is essentially constant over a wide range of temperature but there are varieties whose infection types in response to certain strains of the pathogen may vary from susceptible to more resistant or vice versa, depending upon the postinoculation or even the preinoculation temperature; (2) host reactions 2 and X are the most readily altered ones and types 0,0; and 4 are the most stable. Type X may become only a susceptible or a resistant type under the influence of temperature; (3) shifts in reaction are usually very narrow, from one type to the next one above or below, but a few cases of a highly resistant reaction changing into a highly susceptible type or vice versa have been reported; (4) it appears to take a fairly long time of exposure (a few days) for the temperature to bring about a shift in reaction so that diurnal changes in temperature are not likely to be important in this connection; (5) a shift in reaction response is not permanent but is lost by exposure

of the host to the original temperature for a few days; and
(6) a shift in reaction of a host variety may be induced to
one or more strains but not to other strains.

4. Germinability of Uredospores.

In 1939, Straib observed that the temperature at which
uredospores of P. glumarum, yellow rust of wheat, are
produced determine their germinability. Those produced at
20°-25°C germinated more quickly and over a wider range of
temperatures than did those produced at 8°-10°C.

5. Teliospore Formation

One of the many interesting phenomena in the life
history of a rust is the formation of the teliospores, which
commonly takes place in the sori that previously had produced
uredospores. Many investigators have studied the effect of
environmental factors, especially temperature, light and
moisture on teliospore formation.

Eriksson (1898) suggested that only the teliospores
produced in the late autumn succeed in germinating in the
following spring (Johnson, 1931). Melander (1935) observed
that the production of telia by P. graminis on wheat, oats
and rye was stimulated at a low temperature (0° to 1°C).
Peturson (1930) however, studied the effect of different
environmental conditions on the formation of telia of crown
rust and concluded that telia formed more readily at moderately
warm temperatures than at cool temperatures. Simons (1954)

found that telia of P. coronata avenae were produced earlier and in greater abundance at 25°C, in most cases: this difference was greatest with the more resistant varieties.

Johnson (1931) studied telial formation in physiologic forms of P. graminis tritici. He found that relatively high temperatures favoured rapid teliospore formation, but there were inherent differences also between forms in respect to their telial development. He observed also that most rapid telial development occurred at the nodes of the plants. In other words, environmental factors, constitution of the fungus mycelium and specific host tissues had some effect on teliospore formation. From a study of the telial development of several rust fungi, Waters (1928) concluded that factors such as light, temperature and moisture may so influence the metabolism of the host that the fungus reacts by changing from the uredinial to the telial stage. That is, environmental factors are of importance, but are indirect in their action. Gassner (1915) found that the development of teliospores of the cereal rusts occurred when the host plant reaches a certain stage of maturity, and that telial development is independent of external conditions (Johnson, 1931).

6. Sunflower Rust

The only references in the literature on the temperature relations of P. helianthi that could be found, were those of Bailey (1922, 1923). Both teliospores and uredospores germinate through a rather wide range of temperature, 6° to 28°C, with an optimum at 18°C. Infection by uredospores is

very heavy by the end of an incubation period of twenty-four
hours but some infection can occur in 6 hours. Uredia do
not develop below $10^{o}C$ but the mycelium will remain dormant
in infected leaves for at least a month at this temperature
and uredia will subsequently develop when the plants are
transferred to higher temperatures.

B. LIGHT EFFECTS

In contrast to temperature, which apparently affects
both host and parasite about equally, light as a rule
affects the host more than the pathogen. Light intensity
and quality and length of day may be significant. In general
lack of light for normal photosynthetic activity due to too
short exposure or too low intensity of light tends to weaken
the host making it more susceptible to facultative parasites
and more resistant to obligate ones (Gaumann, 1950).

1. Intensity of Infection

Since the uredospores penetrate through the stomata
much consideration has been given to the significance of the
opening and closing of stomata under the influence of light
as a factor in determining inoculum efficiency. In general,
this appears to be a factor of little or no significance and
most workers use light-tight containers as moist chambers and
obtain good infection (Caldwell and Stone, 1936, Chester, 1946
and Hart and Forbes, 1935). Hart and Forbes (1935) demonstrated
that leaf rust of wheat, crown rust of oats and snapdragon

rust could enter their hosts in darkness as well as in light
while darkness could inhibit infection by the wheat stem
rust fungus and bean rust but only slightly influenced
infection of corn and sunflower rust. Sharp et al. (1958)
obtained maximum inoculum efficiency with the uredospores of
P. graminis tritici at all light intensities below 300 foot-
candles (and within an accompanying temperature range of
$15.6°-23.9°C$)up to the completion of appressoria formation
followed by light intensities over 500 foot-candles (and
higher temperatures).

2. The Incubation Period.

The length of incubation period may be strongly
affected by light. In general relatively high light intensity
and long days are most favourable for rapid invasion and
abundant sporulation. Wheat stem rust develops well under
light intensities ranging from 500 to 10,000 ft-c. The
incubation period is likely to shorten and sporulation to
increase with increasing light (Stakman and Harrar, 1957).
Uredia of Melampsora lini, flax rust, appeared 9 days after
inoculation under normal light, in 6.5 days under continuous
light and in 14 days under reduced light intensity (Hart, 1926).
The incubation period of P. glumarum was twice as long at low
light intensities as it was at 960 ft-c and 9 days longer when
infected barley was exposed to 6-hour days than when it was
exposed to 12-hour days (Bever, 1934). While increase in

light from suboptimal to optimal amounts shortens the
incubation period, Scheibe found that increases above an
optimum amount may cause an extension of this period,
(Chester, 1946).

3. Infection Class and Types

Not only is the incubation period influenced by light
but the type of rust reaction may be altered.   Light is not,
in general, as effective as temperature and host nutrition
in altering reactions. There are many records, however, of
reactions being changed from susceptible to more resistant
or vice versa by changes in the light factor when temperature
is maintained constant (Chester, 1946, Gaumann, 1950, Stakman
and Harrar, 1957).

In 1941, Newton and Johnson tested 7 rust races on 7
differential wheats during 3 months of different light
intensities, and light duration at 60° and 75°F (15.6°-23.9°C).
Many changes from a resistant to a more susceptible type as
light intensity or day length was increased were observed.
This was expressed to a greater or lesser degree by all
varieties toward one or more races and by all races toward one
or more varieties. Varieties normally expressing extreme
resistance or susceptibility were less affected than those
expressing moderate resistance or susceptibility. It was
further seen that temperature was more influential in changing
reaction types than was light; a significant temperature

alteration was observed in cases in which light produced only a slight effect. The normal seasonal variation in the light factor brought about alterations in reactions.

Bever (1934) observed that susceptible varieties of wheat tended to show a more resistant type of infection towards P. glumarum when exposed to day lengths of more than 12 hours. According to Hart and Zaleski (1935) the development of race 21 of wheat stem rust on Hope wheat is depressed by high light intensity while it is normal at lower intensities.

Resistance can be altered by light without a change in infection type. Daly (1964) reported that transfers from incandescent-fluorescent light before inoculation to fluorescent light immediately after incubation increased pustule numbers, whereas the opposite transfer markedly inhibited pustule formation by P. graminis tritici. Transfers following the infection period had the opposite but not so marked effect. He interpreted the data as indicating a major influence of light on the biochemical and physiological factors of the host prior to incubation and on the first day after inoculation.

The increase in resistance of hosts to rusts under inadequate light is generally regarded as being due to the lowered photosynthesis of the host and this contention is supported by the fact that rusts develop on leaves in the dark if they are fed sugar (Chester, 1946).

4. Uredospore Germinability

Uredospores of P. coronata were not viable if they developed on plants lacking sufficient light (Hobbs, 1962).

5. Teliospore Formation

Cereal rusts change from production of uredospores to teliospores when days became short. The uredial stage of U. phaseoli continues to develop when daylight hours equal or exceed the hours of darkness but when nights became longer than the day, then, only the telial stage appears; in Florida the telial stage never appears (Chupp and Sherf, 1960).

6. Sunflower Rust

Bailey(1923) found that light had a marked influence on the rapidity of the development of sunflower rust. Reduced light increased the length of the incubation period by about 2 days and a further reduction of the light intensity appeared to prolong it indefinitely.

III MATERIALS AND METHODS

Sunflower rust races 1 and 3 (Sackston, 1962) were used in these studies. Two varieties of sunflower plants were used: S37-388, which is susceptible to all collections of rust from Helianthus annuus so far tested; and CM90RR which is resistant to race 1 but susceptible to race 3. Seed of these two varieties was supplied by Dr. E.D Putt, Canada Department of Agriculture Experimental Farm, Morden, Manitoba.

Seedlings were grown in 4-inch pots in a greenhouse maintained at 70°F (21.1°C) in winter, fluctuating temperatures in spring and summer. Six to seven seeds were sown in each pot. When the first pair of true leaves were fully expanded, three to five plants in each pot were chosen for uniformity of size and vigor and the rest were discarded. In most experiments, seedlings were inoculated when the second pair of leaves was well unfolded.

Plants were inoculated with uredospores suspended in distilled water with 0.1% Tween-80 to reduce surface tension, sprayed from a deVilbiss atomizer. After inoculation, plants were incubated in a saturated light-tight moist chamber kept at approximately 20° to 23°C for 24 hours. At the end of 24 hours, plants were incubated in controlled environment chambers.

Uredospores were collected when the rust pustules reached full development by shaking the leaves with tweezers into petri dishes. Germinability of fresh spores produced

under different conditions was determined on spores collected.
The remainder was kept in the freezer (-16° to -20°C) for
several weeks before being used, to determine *the effect* on germinability
of stored spores. Spores were distributed uniformly on 1%
water agar either on standard slides or in 9 cm petri dishes
by means of an aspirator, as shown in Figure 1. They were
incubated at room temperature in darkness. Results were record-
ed 24 hours after inoculation. The spores were covered with
a drop of lactophenol and cotton blue and a coverslip deposited
before counting. The germination percentage data were analyzed
statistically using the Arcsin transformation (Steel and
Torrie, 1960).

The reaction types were classified by size and vigor of
sporulation of uredia as indicated by Sackston (1962):

0; - Hypersensitive flecks.

1 - Very resistant, producing very small, very
weakly sporulating uredia.

2 - Moderately resistant, producing small, less
strongly sporulating uredia.

3 - Moderately susceptible, producing medium-sized,
vigorously sporulating uredia.

4 - Very susceptible, producing large, vigorously
sporulating uredia.

The statistical design used for most experiments was the
randomized complete block. For the individual analyses of
treatments, Duncan's new multiple-range test was used (Steel
and Torrie, 1960).
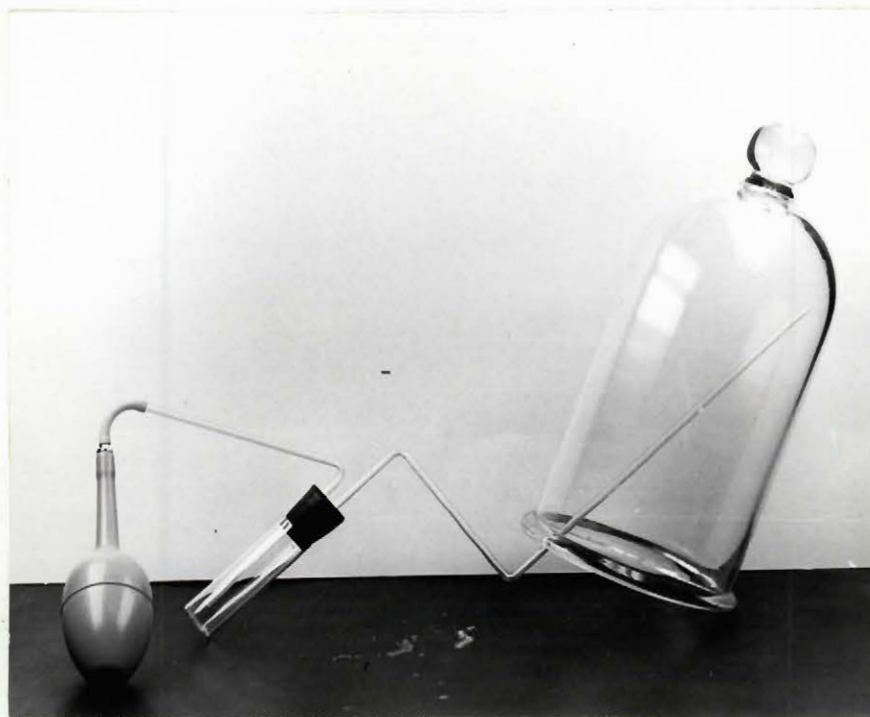
Fig. 1.   Aspirator for uniform distribution
           of rust spores to determine
           germinability.

IV. EXPERIMENTAL RESULTS

A. TEMPERATURE EFFECTS

On August 8, 1964, two varieties of sunflower plants
(S37-388 and CM90RR) were sown in 4-inch pots and grown in
the greenhouse. Twenty-four pots of each variety, each
with 3 plants, were selected according to the uniformity of
the plants. Random groups of pots of each variety were
placed in each of 4 controlled environment chambers maintained
at 15°, 20°, 25°, and $30^{\circ}C$ respectively, with 8 pots at 15°
and $20^{\circ}C$, and 4 pots at 25° and $30^{\circ}C$. The plants in each
chamber were illuminated with 1500 ft-c of cool white fluorescent
light with photoperiod of 16 hours. At the end of 3 days
the plants were removed from the chambers. Half of the pots
of each variety from each chamber were inoculated with race 1
and the other half were inoculated with race 3. The spore
mass was suspended in 0.1% Tween 80, with 4 mg of spores
per ml. After inoculation the plants were placed in a light-
tight moist chamber at room temperature ($21^{\circ}C$) for 24 hours.
At the end of this period, 2 pots of each race-variety
combination were exposed to the following regimes:

Pre-inoculation temperature	Post-inoculation temperature
$15^{\circ}C$	$15^{\circ}C$
$15^{\circ}C$	$20^{\circ}C$
$20^{\circ}C$	$15^{\circ}C$
$20^{\circ}C$	$20^{\circ}C$
$25^{\circ}C$	$25^{\circ}C$
$30^{\circ}C$	$25^{\circ}C$

The experimental results, unfortunately, were not recorded properly, and the concentration of spore suspension proved to be too high. However, it was observed that the density of pustules was greater and the reaction type developed better under the $15^{\circ}C$ pre-inoculation and $20^{\circ}C$ post-inoculation temperature regime than at 15° and $15^{\circ}C$, 20° and $20^{\circ}C$, and 20° and $15^{\circ}C$ respectively. Pre-inoculation temperature of $30^{\circ}C$ and post-inoculation $25^{\circ}C$ gave better results than the 25° and $25^{\circ}C$ regime.

The following experiments were designed to provide data on the influence of constant temperatures, varying day-night temperatures, and various pre- and post-inoculation temperature regimes on sunflower rust development.

1. Constant Post-infection Temperatures

An experiment was designed to provide data upon the effects of constant post-infection temperatures upon (a) inoculum efficiency as appraised by the density of rust pustule development, (b) the length of the incubation period or time from inoculation to appearance of rust symptoms, (c) type of pustule, (d) uredospore germinability, and (e) telial formation.

Sunflowers of two varieties (S37-388 and CM90RR) were sown on February 1st, 1965, and grown in the greenhouse. Twenty-four pots of each variety (each with 3 plants with second pair of leaves fully developed) were selected for

uniformity of plants and inoculated on March 4th, 1965. Twelve pots of each variety were uniformly inoculated with a uredospore suspension of race 1, and the remaining 12 pots of each variety were inoculated with race 3. One ml of suspension contained approximately 2 mg of fresh spores.

The inoculated plants were placed in a light-tight moist chamber in a room kept at 21° - $23^\circ C$. After 24 hours, 3 pots of each variety-race combination were transferred to each of four controlled environment chambers maintained at 15°, 20°, 25°, and $29^\circ C$, respectively. The light period was 16 hours per day, at 1500 foot candles. The plants were observed daily for 15 days.

a) <u>Inoculum efficiency as determined by density of</u>
<u>pustules</u>

The density readings (number of rust pustules per square centimeter of leaf surface) were made 15 days after inoculation. The averages of 3 random readings for each set of three plants are recorded in Table 1.

The data were analyzed statistically. There was no significant difference between older and younger leaves (Appendix Table I, II and III). When S37-388 was inoculated with race 1 the interaction between leaf age and temperature was very highly significant. Duncan's multiple-range test was applied to the data for infection on leaves of two ages at the various temperatures. The younger leaves (second pair) were more sensitive than older leaves to the influence of temperature.

TABLE 1.   Effect of constant post-infection temperature on density of rust pustules per cm^2 of leaf surface (No.) and on host reaction type (Type) of sunflowers.

Variety	Temperature °C	Race 1				Race 3			
		1st leaves		2nd leaves		1st leaves		2nd leaves	
		No.[1]	Type	No.	Type	No.	Type	No.	Type
S37-388	15°	19.7	$2^-,2$	7.3	$2^+,3$	14	$2^-,2$	16	$3^-,3$
	20°	16	$3^-,3$	15	$3^-$to 4	13	3.4	15.7	3.4
	25°	16.7	$3^-$to 4	15.7	3.4	16.7	$3^-$to 4	16	$3^-$to 4
	29°	13.7	$3^-,3$	7.3	$3^-$to 4	-	-	-	-
CM90RR	15°	0	0;	0	0;	12	$1^+,2$	10.7	$1^+$to 2^+
	20°	0	0;	0	0;	15.7	3	18.3	$3^-$to 4
	25°	0	0;	0	0;	21	3,4	22.7	$3^-$to 4
	29°	0	0;	0	0;	9	$2^+,3^-$	6.3	$2^-$to 3

1) Average readings for the leaves of 9 plants (3 pots, 3 plants per pot).

There were no significant differences in pustule
density at various temperatures of either pair of leaves of
S37-388 inoculated with race 3 (Figures 2 and 3). Pustule
density on younger leaves inoculated with race 1 was
significantly greater at 20° and $25^{\circ}C$ than at 15° and $29^{\circ}C$
(Appendix Tables I-a and I-b).

On CM90RR inoculated with race 3, pustule density was
significantly higher on older leaves at $25^{\circ}C$ than at $29^{\circ}C$
(Fig. 4, Appendix Tables II-a and II-b). On younger leaves
density was significantly higher at 20° and $25^{\circ}C$ than at 15°
and $29^{\circ}C$ (Fig. 5).

b) <u>Length of incubation period</u>

The effect of the four post-infection temperatures upon
the length in days of the incubation period or time from
inoculation to earliest appearance of symptoms is shown in
Fig. 6. The incubation period was shortest at $25^{\circ}C$ for both
races on the two varieties. The incubation periods for both
races were consistantly somewhat shorter on variety CM90RR than
on S37-388 at 15°, 20° and $25^{\circ}C$ but at $29^{\circ}C$ they were
practically the same.

The length of time required for sporulation of both
races on variety S37-388 and of race 3 on CM90RR at 15°, 20°,
25° and $29^{\circ}C$ was 10, 7-8, 7, and 7 days respectively.

c) <u>Type of pustule</u>

Data concerning the influence of the four post-infection
temperatures upon host reaction type are included in Table 1.

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Fig. 2   Effect of constant temperature on first
          leaves of S37-388 inoculated with race 3.
          From left to right 25°, 20°, and 15°C,
          respectively.

Fig. 3   Effect of constant temperature on second
          pair of leaves of S37-388 inoculated
          with race 3. From left to right 25°, 20°,
          and 15°C, respectively.
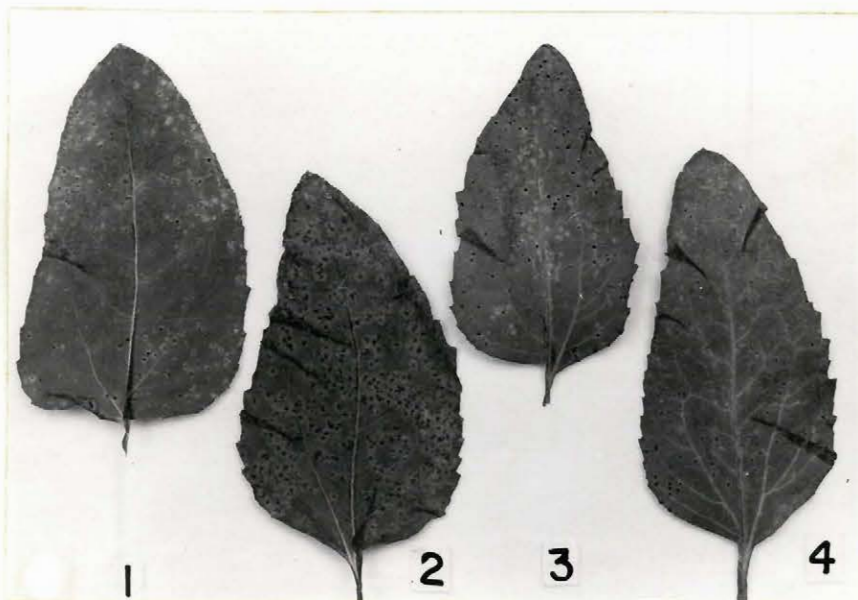
Fig. 4 Effect of constant temperature on first
        leaves of CM90RR inoculated with race 3.
        No. 1, 2, 3 and 4 indicates 29°, 25°,
        20° and 15°C respectively.
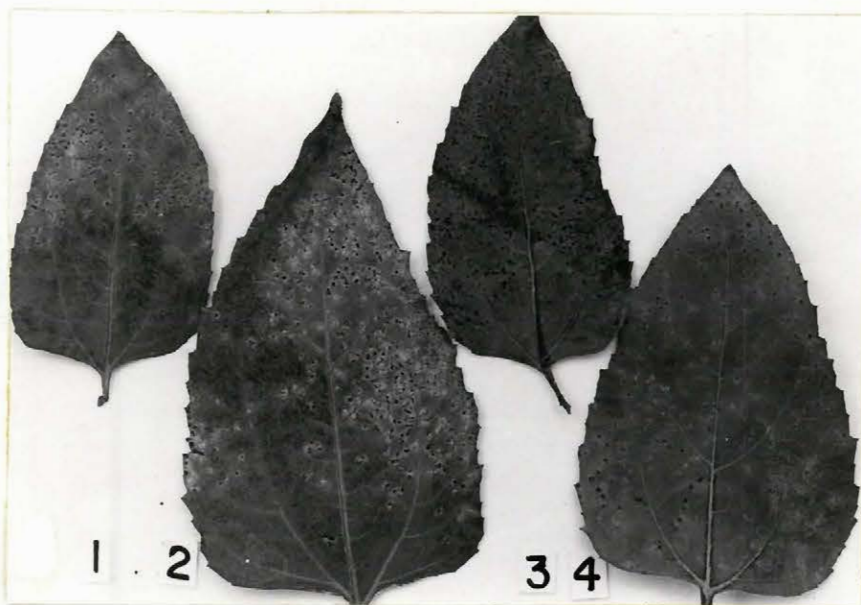
Fig. 5 Effect of constant temperature on second
        pair of leaves of CM90RR inoculated with
        race 3. No. 1, 2, 3 and 4 indicates 29°,
        25°, 20° and 15°C respectively.
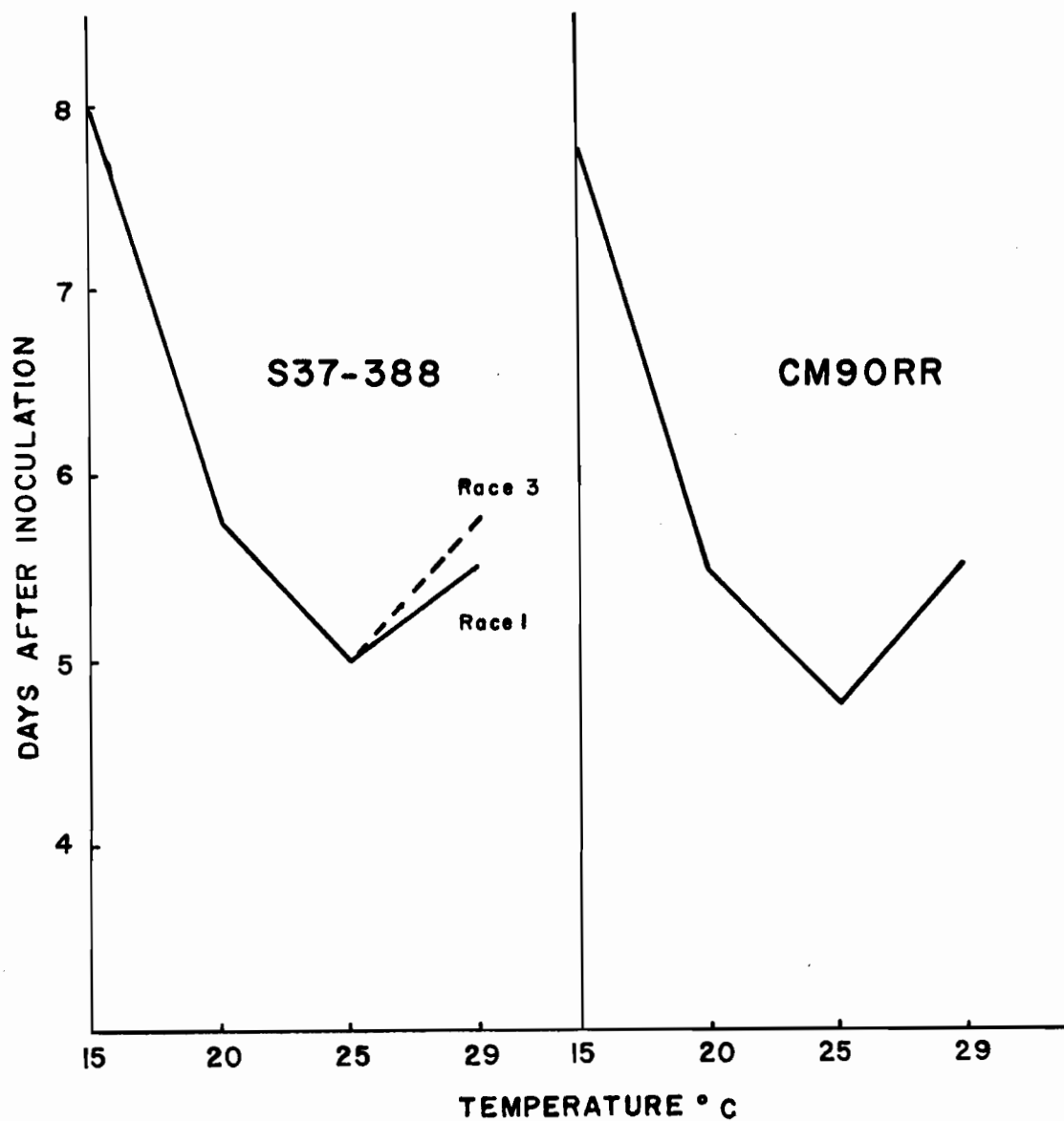
Fig. 6   Effect of constant post-infection temperature
          upon the length of incubation period of
          <u>Puccinia helianthi</u>, races 1 and 3 on varieties
          S37-388 and CM90RR.

Temperature had no influence on the resistant reaction (type 0;) resulting from race 1 on CM90RR. The susceptible 3 and 4 type reactions induced by race 1 and 3 on S37-388, and race 3 on CM90RR, proved to be influenced by temperature variation. They showed some, usually small, difference in reaction type as the temperature varied. Rather striking difference in reaction type on the first leaves from 3 and 4 at 25° to 1+ and 2 at 15°C occurred with race 3 on CM90RR (Fig. 4). In general changes in reaction type were more pronounced on lesions on the upper leaf surface than on the lower surface. Differences among reaction types under various conditions tended to get less with time, approximately 20 days after inoculation.

d) Uredospore germinability

Spores produced by race 1 on S37-388 and by race 3 on CM90RR at 15°, 20°, 25° and 29°C were collected for germinability tests 12 days after inoculation. Half the volume of spores was used to determine germinability of the freshly harvested spores at room temperature on 1% water agar while the remaining half was stored at -16°C for 60 days and then subjected to a similar germinability test.

The data for germination of freshly harvested spores are given in Appendix Tables IV and V. In Figure 7 it can be seen that spores of race 1 gave very high germination irrespective of temperature of production. The statistical analyses of these data given in Table 2 reveal no significant differences in the germination percentages. The percentage
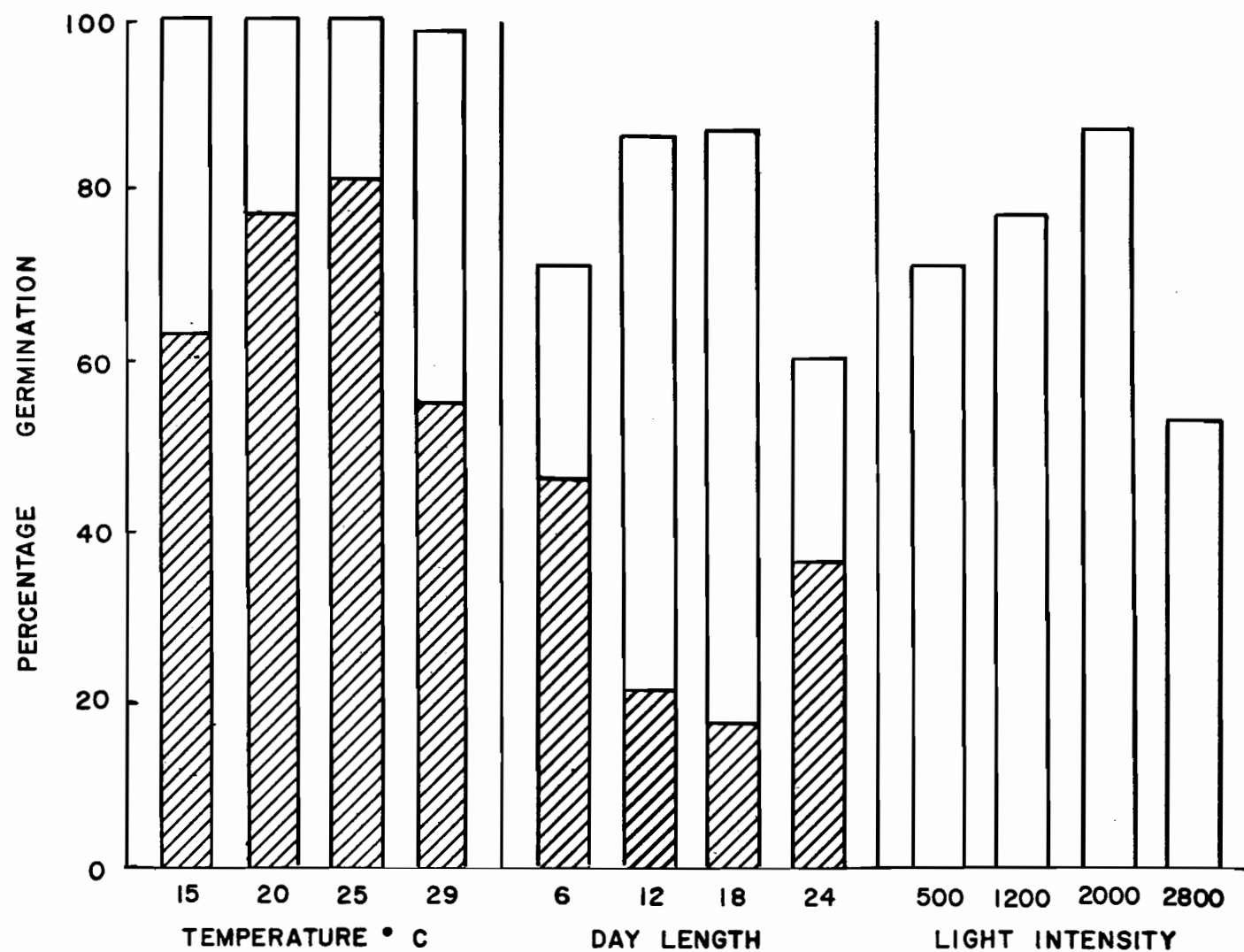
Fig. 7   Effect of conditions during spore production on germinability of
fresh and stored uredospores of _Puccinia_ _helianthi_ race 1.

Unshaded portion of bar in each case represents fresh spores,
shaded portion spores stored 60 days at -16°C.
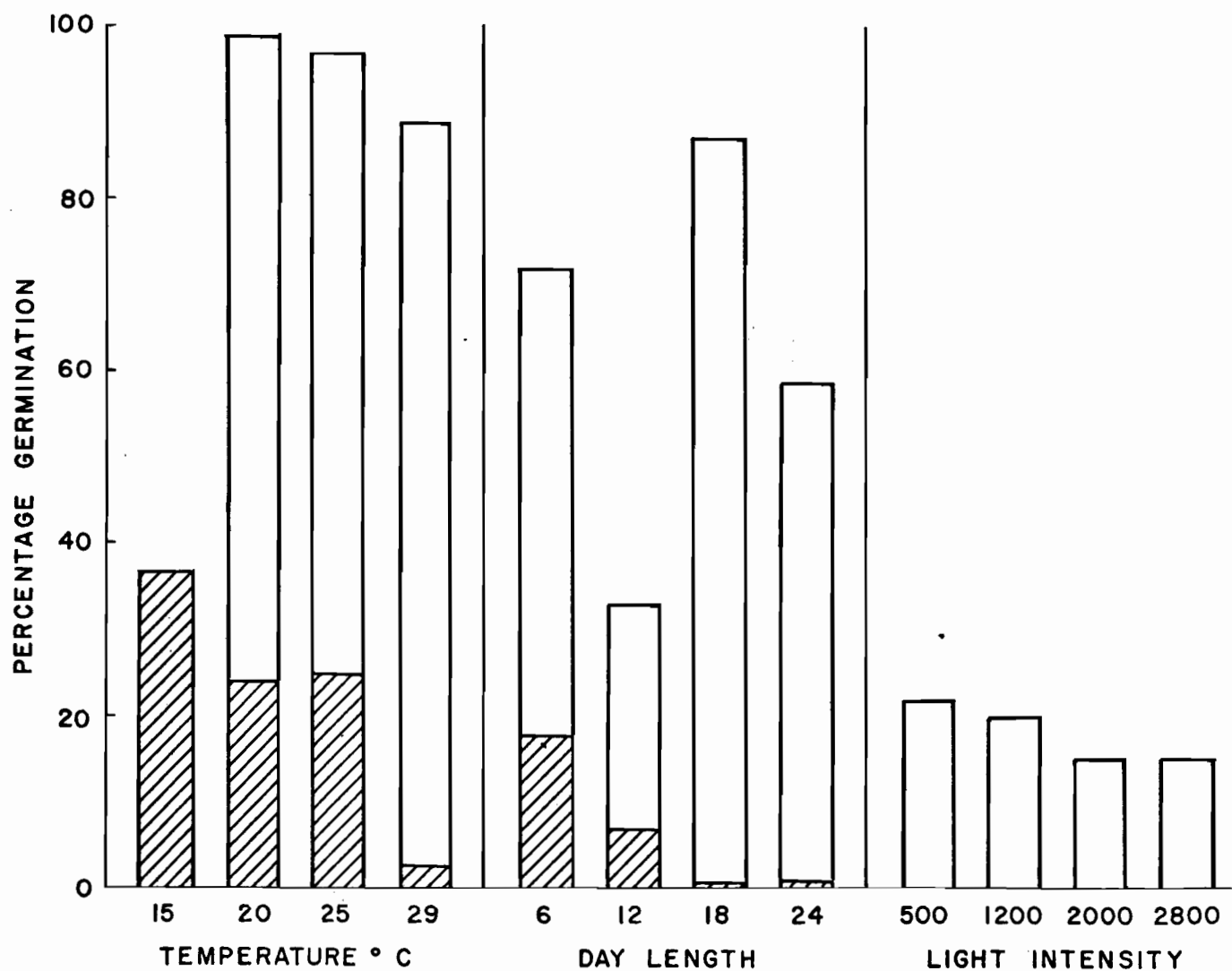
Fig. 8 Effect of conditions during spore production on germinability of fresh and stored uredospores of <u>Puccinia helianthi</u> race 3.

Unshaded portion of bar in each case represents fresh spores, shaded portion spores stored 60 days at -16°C.

TABLE 2.   Duncan's new multiple-range test for
            the effect of post-infection
            temperatures in which uredospores were
            produced upon the percentage germin-
            ation of fresh uredospores.

Race 1 on Variety S37-388

Temperature	15°C	20°C	25°C	29°C
Average of percentage[1] germination	100	100	99	99

Race 3 on Variety CM90RR

Temperature	20°C	25°C	29°C
Average of percentage[2] germination	99	97	89

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1) Any two means underscored by the same line are
   not significantly different.

2) Any two means not underscored by the same line
   are significantly different.

germination of spores of race 3 produced at 20°C was significantly higher than those produced at 25° and 29°C. Not enough spores of race 3 were produced at 15°C to determine germination percentage $(Fig. 8)$.

The data for the germination of the stored spores are given in Appendix Tables VI and VII. The storage conditions reduced markedly the germinability of the spores of both races. The reduction was more pronounced with race 3 than with race 1. The statistical analyses presented in Table 3 show that the spores of race 1 produced at 20° and 25°C retained significantly higher germination values than did those produced at 15° and 29°C. On the other hand, the spores of race 3 produced at 15°C retained a significantly higher capacity to germinate than did those produced at the other 3 temperatures. Germination of spores produced at 29°C fell to very low levels.

e) Teliospore formation and infectivity

As soon as sporulation began, spores were harvested daily by lightly scraping different pustules each day with the moistened blade of a scalpel. The spores were suspended in distilled water and examined under the microscope for the occurrence of teliospores.

Table 4 gives the length of time it took the teliospores of race 1 to form on variety S37-388 and of race 3 on variety CM90RR whereas those of race 3 on S37-388 were not recorded. It can be seen that, at all temperatures, race 1 on S37-388 formed teliospores earlier than race 3 on CM90RR. Teliospores of both races occurred earliest at 15°C.

TABLE 3. Duncan's new multiple-range test for the effect
of post-infection temperature in which
uredospores were produced upon the percentage
germination of stored spores.

Race 1 on Variety S37-388

Temperature	25°C	20°C	15°C	29°C
Average of percentage germination	81	77	63	55

Race 3 on Variety CM90RR

Temperature	15°C	25°C	20°C	29°C
Average of percentage germination	37	25	24	3

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TABLE 4.   The effect of post-infection temperature on
             formation of teliospores on sunflower rust.

| Variety | Race | First occurrence of teliospores (days after inoculation) | | | |
		15°C	20°C	25°C	29°C
S37-388	1	16	16-17	18-19	19-20
CM90RR	3	17	17-18	21-22	23-24

Teliospore formation was more abundant at 15° and 20°C than at 25° and 29°C.

Approximately 1 month after inoculation rusted leaves bearing teliospores produced at various temperatures were harvested and stored at 6°C to maintain the vitality of the telio- spores as well as to break spore dormancy. After 25 days, leaves were removed from cold storage and alternately soaked all day and dried overnight for 3 or 4 times to induce germin- ation. The treated telia-bearing leaves were then used for inoculating healthy sunflower plants. This was accomplished by placing a leaf on a moist filter paper in the bottom of a petri dish which was then inverted and placed as a cover on the top of a lamp chimney positioned on a flower pot so as to enclose the 6 sunflower plants growing in it. Three pots of plants were inoculated with teliospores of each race produced on the two varieties at each of four temperatures. The pots were placed in a light-tight moist chamber for 48 hours at 20°C. The plants were then removed, uncovered, and placed in a controlled- environment chamber maintained at 20°C and illuminiated with 1500 ft-c at plant level for a period of 16 hours per day.

The number of pycnia on all plants was recorded 20 days after inoculation (Table 5). Teliospores of races 1 and 3 on S37-388 and of race 3 on CM90RR produced at 15°C induced numerous infections. Those produced at 20°C induced few infections. No infections were induced by the teliospores produced under the other conditions.

TABLE 5.   The effect of temperature at which

teliospores were formed on their

infectivity.

Temperature ^{o}C	Number of pycnia 20 days after inoculation.			
	S37-388		CM90RR	
	Race 1	Race 3	Race 1	Race 3
15°	16	37	0	9
20°	5	0	0	4
25°	0	0	0	0
29°	0	0	0	0

2. Varying Day-Night Post-infection Temperatures

This experiment was carried out to study the effects of
various artificial day and night temperature regimes or
profiles, (simulating, at least to some degree, the range of
natural diurnal temperature changes) upon (a) inoculum
efficiency as determined by the density of pustule development,
(b) the length of the incubation period, and (c) type of
pustule formed.

Plants for this experiment were sown April 7 and
inoculated May 4 in the routine way. The spore suspension
contained approximately 1 mg of spores (collected and stored
on April 19, 1965) per ml. After incubation for 24 hours in a
moist chamber in the dark, 3 pots of each variety inoculated
with each race were placed in controlled environment chambers
with 1500 ft-c illumination for 16 hours per day (6 a.m. to
10 p.m.) and the following temperature programs:

	Day	Night
1.	25°C	25°C
2.	25°C	18°C
3.	33°C	23°C
4.	28°C 6 a.m. - 10 a.m.	
	33°C 10 a.m. - 6 p.m.	
	28°C 6 p.m. - 10 p.m.	
	25°C 10 p.m. - 6 a.m.	

The temperature mechanisms of these chambers were very efficient
and adjusted the air temperature from one level to another in

15 minutes or less depending upon the magnitude of the change required. The stepwise temperature regime (4) was included to approach more closely the gradual and rather long drawn out diurnal cycle of temperature changes occurring under natural conditions.

a) <u>Inoculum efficiency as determined by density of</u>
<u>pustules</u>

The number of pustules on half of each leaf surface was counted 14 days after inoculation. The results are shown in Table 6.

The data were analyzed statistically (Appendix Tables VIII, IX and X). Differences due to temperature were not significant for race 1 on S37-388, highly significant for race 3 on S37-388, and significant at the 5% level for race 3 on CM90RR. Differences due to age of leaves were significant at the 5% level (race 1 on S37-388) or the 1% level (race 3 on S37-388 and on CM90RR). There was a significant interaction between age of leaves and temperature regime in S37-388 inoculated with race 1, and CM90RR inoculated with race 3. Duncan's multiple range test was applied to the means for individual variety-race combinations exposed to the various temperature regimes (Appendix Tables VIII-a, VIII-b, IX-a, IX-b, X-a, and X-b). Age of leaves of S37-388 inoculated with race 1 was significant only in the 25° -25°C and 28°-33°- 28°-25°C regimes (Appendix Table VIII-c). Age of leaves of CM90RR was significant in all temperature regimes (Appendix Table X-c).

TABLE 6. Effect of varying post-infection temperature on density of rust pustules per half leaf (No.) and on host reaction type (Type) on sunflowers.

Variety	Day-night Temperatures °C	Race 1				Race 3			
		1st leaves		2nd leaves		1st leaves		2nd leaves	
		No.[1]	Type	No.	Type	No.	Type	No.	Type
S37-388	25° – 25°	95.6	2 to 4	67.8	3^- to 4	89.0	2 to 4	57.6	3^- to 4
	25° – 18°	78.8	2^- to 4	64.5	3^- to 4	95.2	2 to 3	86	3^- to 4
	33° – 23°	56.6	1 to 2^-	53.1	2^- to 3	63.9	1^+ to 2	42.8	2 to 3
	28°-33°- 28°-25°	96.8	1^+ to 3	73.4	2 to 4	78.8	2^- to 2^+	65.5	2^+ to 4
CM90RR	25° – 25°	1	2	0.2	2	77.9	3^- to 4	30.5	3^- to 4
	25° – 18°	1	2^-	0.3	2^-	99.8	2^- to 4	61.4	2^+ to 4
	33° – 23°	0.1	2^+	0.1	2	52.8	1^+ to 2	24.8	1^+ to 3^-
	28°-33°-28°-25°	0.4	2^+	0	–	83.4	2^- to 3	58.5	2^+ to 4

1) Average readings for the leaves of 12 plants (3 pots, 4 plants per pot)

b) <u>Length of incubation period</u>

The effect of the four day-night post-infection temp-
erature regimes upon the length in days of the incubation
period is shown in Fig. 9. The incubation period is 5 days at
25°C day-night for both varieties infected with either of the
two races. At 33°C day and 23°C night temperatures the
incubation period is 6 days for these race-variety combin-
ations. At 25°C day and 18°C night, the incubation period on
CM90RR inoculated with either race was 1 day shorter than on
S37-388 inoculated with either race. Under the stepwise temp-
erature regime (28°-33°-28°-25°C) the incubation period for
race 1 was 1 day shorter than for race 3 on S37-388, and 1 day
longer than for race 3 on CM90RR.

Sporulation of both races on variety S37-388 and of
race 3 on variety CM90RR at 33°C day and 23°C night occurred 2
days after flecks appeared. Under all other conditions
sporulation occurred 1 day after the appearance of flecks.

c) <u>Type of pustule</u>

The effect of day-night post-infection temperature
regimes upon the type of pustules is shown in Table 6. The day-
night temperature regimes had no marked influence on the
resistant reaction of variety CM90RR infected by race 1. The
susceptible type 3 and 4 reactions on S37-388 infected with
either race, and on CM90RR infected with race 3 appeared to be
affected more by the day-night temperature variations (Figs. 10,

S37-388

CM9ORR

Race 1 Race 3 Race 1 Race 3

DAYS AFTER INOCULATION

6

5

4

| DAY | 25 | 25 | 28-33-28 | 33 | 25 | 25 | 28-33-28 | 33 | 25 | 25 | 28-33-28 | 33 | 25 | 25 | 28-33-28 | 33 |
| NIGHT | 18 | 25 | 25 | 23 | 18 | 25 | 25 | 23 | 18 | 25 | 25 | 23 | 18 | 25 | 25 | 23 |

TEMPERATURE ° C

Fig. 9. Effect of day-night post-infection temperature upon the length of incubation period of <u>Puccinia helianthi</u> races 1 and 3 on varieties S37-388 and CM9ORR.

Fig. 10   Effect of day-night temperature on
first pair of leaves of S37-388
inoculated with race 1. From left
to right 25°-18°C, 25°-25°C,
28°-33°-28°-25°C, and 33°-23°C
respectively.

Fig. 11   Effect of day-night temperature on
second pair of leaves of S37-388
inoculated with race 1. From left to
right 25°-18°C, 25°-25°C,
28°-33°-28°-25°C, and 33°-23°C
respectively.

Fig. 12   Effect of day-night temperature on first pair of leaves of CM90RR inoculated with race 3. From left to right $25^{\circ}-18^{\circ}C$, $25^{\circ}-25^{\circ}C$, $28^{\circ}-33^{\circ}-28^{\circ}-25^{\circ}C$ and $33^{\circ}-23^{\circ}C$, respectively.

Fig. 13   Effect of day-night temperature on second pair of leaves of CM90RR inoculated with race 3. From left to right $25^{\circ}-18^{\circ}C$, $25^{\circ}-25^{\circ}C$, $28^{\circ}-33^{\circ}-28^{\circ}-25^{\circ}C$ and $33^{\circ}-23^{\circ}C$ respectively.

11, 12 and 13). Young leaves of S37-388 inoculated with
race 1 showed strikingly less chlorosis under the 33°-23°C
regime than under the other conditions (Fig. 11).

The reaction type of both races on variety S37-388
is lowest at 33°C day with 23°C night. Race 3 on CM90RR
induced some flecks at all temperature conditions, though
many type 3 and 4 reactions were present on the same leaf
surface.

At all times, rust pustules were more numerous and
larger on the lower leaf surfaces than on the upper ones,
of both the first and second pairs of leaves. The reaction
types on the lower leaf surfaces were usually 1 class
higher (more susceptible) than those on the upper surfaces.

3. <u>Pre-inoculation and Post-inoculation Temperatures</u>

This experiment was an attempt to provide data on the
effects of pre-inoculation and post-infection temperatures
upon (a) inoculum efficiency as determined by density of
pustules, (b) the length of the incubation period and (c) type
of pustule.

On February 17, 1965, seeds of the two varieties,
S37-388 and CM90RR were sown in 4-inch pots. On March 19 when
the second pair of leaves had attained their full expansion,
14 pots of S37-388 and 28 pots of CM90RR were selected for
uniformity of the plants. Four pots of S37-388 and 8 of CM90RR
were placed in each of 3 controlled environment chambers main-
tained at 15°, 20°, and 29°C respectively. Two pots of S37-388
and 4 of CM90RR were placed in a chamber operating constantly
at 24°C. The temperature variations in these chambers did not
exceed $\pm 1^{\circ}$C. The plants in each chamber were illuminated with
1500 ft-c of white fluorescent light; the photoperiod was 16
hours. At the end of 3 days the plants were removed from the
chambers. S37-388 was inoculated with race 1; half of the
pots of CM90RR from each chamber were inoculated with race 1,
and the other half from each chamber with race 3. The spore
suspension used as inoculum contained 1 to 1.5 mg of uredospores
(collected and stored on January 29, 1965) per ml. After
inoculation the plants were placed in a light-tight moist
chamber at room temperature (21°-23°C) for 24 hours. Upon
removal from the moist chamber, 2 pots of each variety inoculated

with each race were returned to the chambers in which they had
been conditioned prior to inoculation.   Another 2 pots of
each variety-race combination were placed under new conditions,
as follows:

Pre-inoculation temperature	Post-inoculation temperature
15°C	24°C
20°C	29°C
29°C	15°C

The plants were observed daily.

a) Inoculum efficiency as determined by density of pustules

The number of pustules per square centimetre of leaf
surface was counted 13 days after inoculation.   The average
readings for each set of 2 pots (8 plants) of S37-388
inoculated with race 1 and CM90RR with race 3 are presented in
Table 7.   Plants of CM90RR inoculated with race 1 showed only
flecks under all conditions;   no pustules were produced.

The analyses of variance of the data for S37-388
inoculated with race 1 are shown in Appendix Table XI.   Diff-
erences due to age of leaves and to pre-inoculation and post-
infection temperatures were not significant.   Furthermore, the
interaction between age of leaves and pre- and post-inoculation
temperature was also non-significant.

Analysis of the data for CM90RR inoculated with race 3
(Appendix Table XII) showed no significant differences due to
temperatures or to age of leaves, but a significant interaction

TABLE 7.   Effect of pre-inoculation and post-inoculation temperatures on density of rust pustules per cm^2 of leaf surface (No.) and on host reaction type (Type) of sunflowers.

Variety	Temperature °C Pre-inoc.	Post-inoc.	Race 1 1st leaves No.[1]	Type	2nd leaves No.	Type	Race 3 1st leaves No.	Type	2nd leaves No.	Type
S37-388	15°	15°	17.2	1^+ to 2	15.7	2, 2^+				
	20°	20°	10.8	2^+ to 3	19.0	3, 3^+				
	24°	24°	12.7	3^-, 3	14.3	3^- to 4				
	15°	24°	11.3	3, 3^+	15.0	3, 4				
	20°	29°	11.5	3^-, 3	17.3	3^-, 3				
	29°	15°	15.2	1^+ to 2	10.5	2^-, 2				
CM90RR	15°	15°					11.3	1^+ to 2	25.8	2, 2^+
	20°	20°					20.7	2^+ to 3	16.7	3^-, 3
	24°	24°					15.2	2^+ to 3	11.5	3, 4
	29°	29°					2.8	2, 2^+	6.8	3^- to 4
	15°	24°					19.8	3, 4	17.2	3, 4
	20°	29°					8	3^-, 3	6.8	3^-, 3
	29°	15°					10.5	1^+, 2^-	6.7	1^+, 2^-

1) Average readings for the leaves of 8 plants (2 pots, 4 plants per pot).

between leaf age and temperature regime. When Duncan's new
multiple-range test was applied to the data (Appendix Tables
XII-a and XII-b), there was no significant difference in
pustule densities on the first (older) leaves exposed to the
20°-20°C, 15°-24°C, and 24°-24°C regimes, but the 20°-20°C
and 15°-24°C regimes gave significantly higher pustule
densities than the other temperature combinations. Pustule
densities were lowest on first leaves of plants exposed to
the 29°-29°C regime. On the younger second leaves, highest
pustule densities developed in the 15°-15°C regime, and the
lowest in 29°-15°C, 29°-29°C, 20°-29°C and 24°-24°C regimes.

b) Length of incubation period

The effect of the pre-inoculation and post-infection
temperatures on length of the incubation period is shown in
Figure 14. There were no differences in incubation period
attributable to host variety or rust race in any temperature
sequence.

The shortest incubation period, 4 days, was observed
in plants exposed to the sequences 24°-24°C, and 20°-29°C.
The longest incubation period, 7 to 8 days, was observed in
the sequence 15°-15°C (which gave the highest density of
rust pustules on second leaves of CM90RR inoculated with race
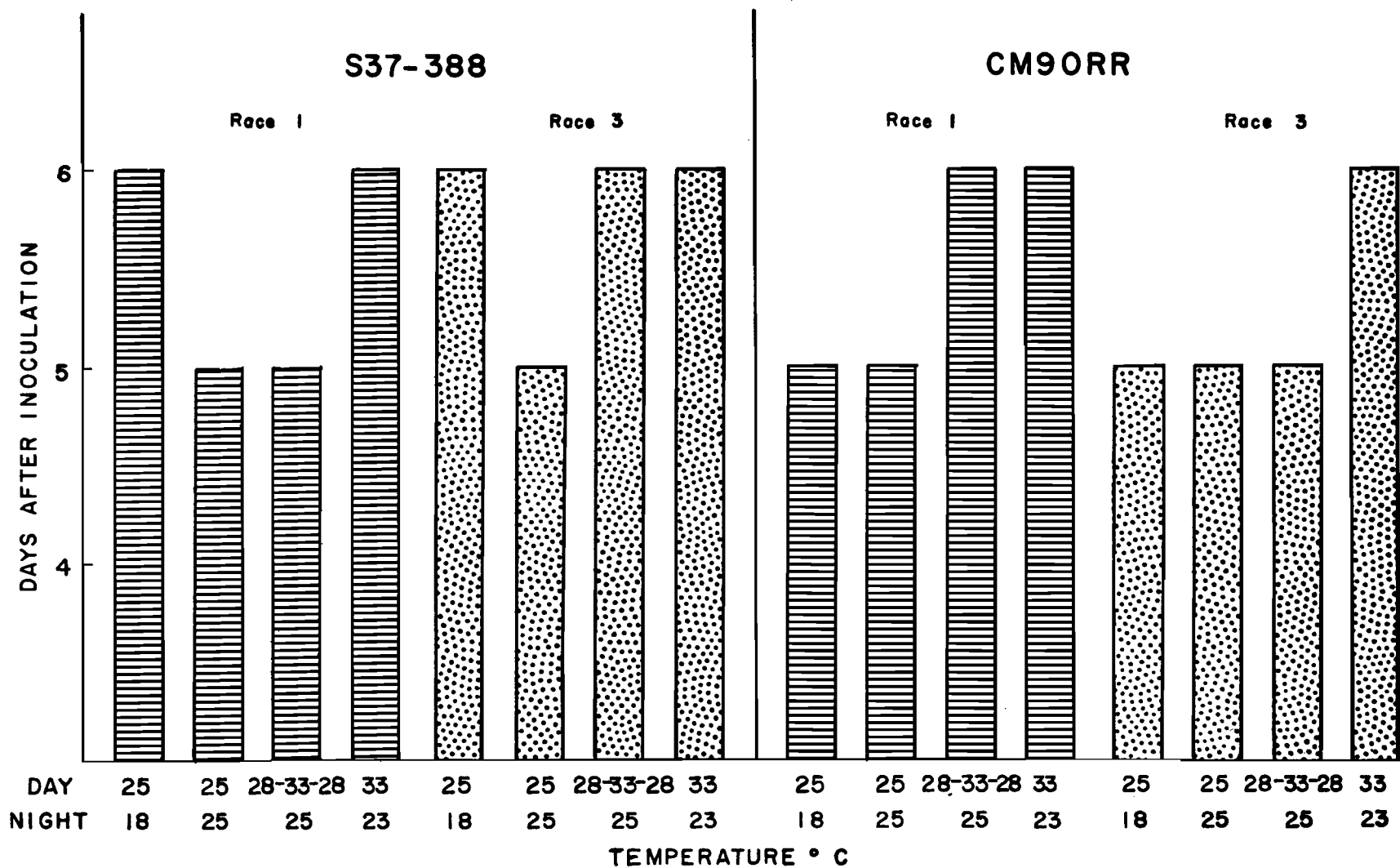3 - Table 7).
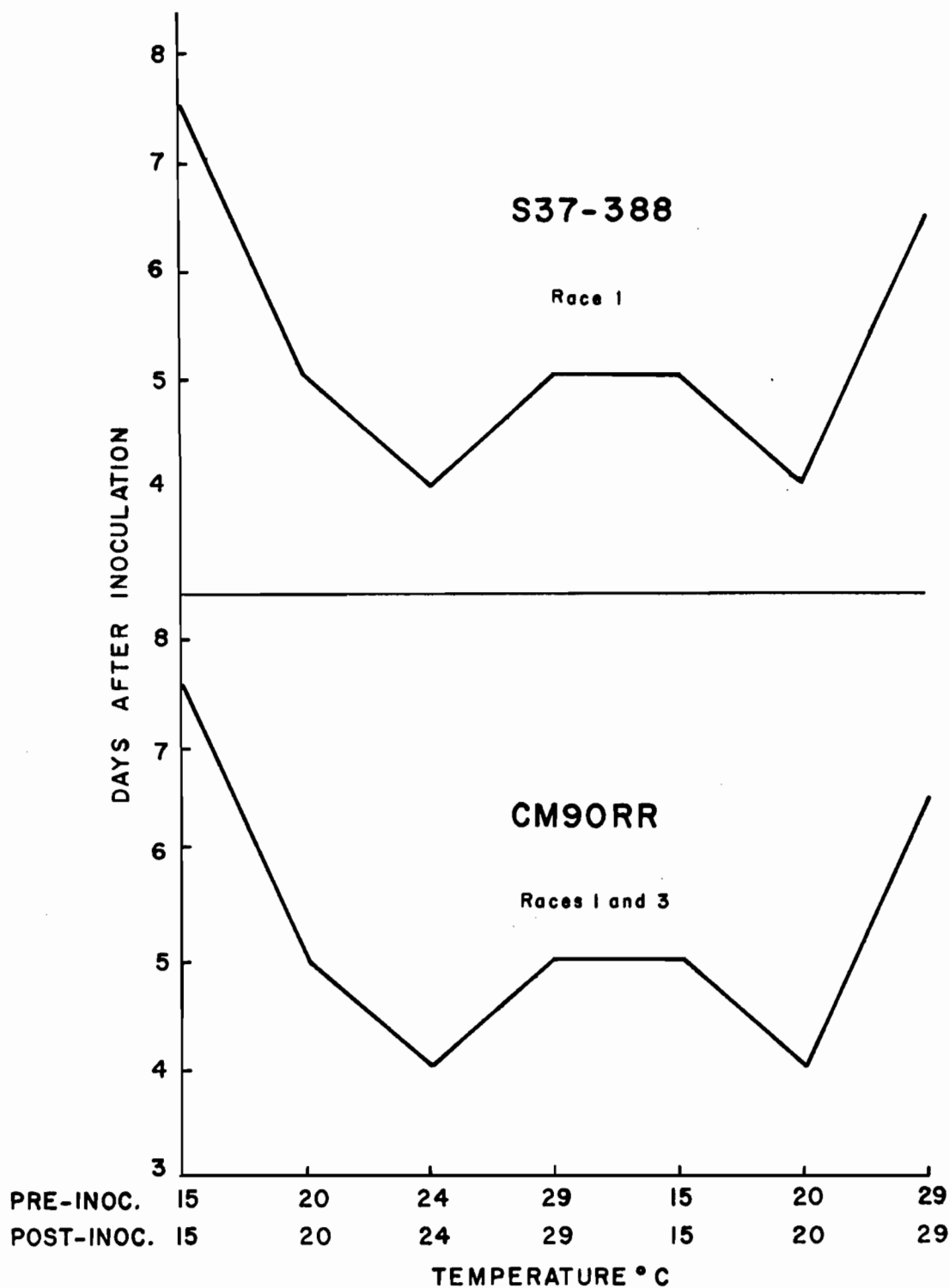
Fig. 14.   Effect of pre- and post-inoculation temperature
           upon the length of the incubation period of
           <u>Puccinia helianthi</u>, race 1 on variety S37-388
           and races 1 and 3 on variety CM90RR.

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c) <u>Type of pustule</u>

The effect of pre-inoculation and post-infection
temperatures on reaction types is shown in Table 7. S37-388
gave susceptible reactions to race 1 and CM90RR to race 3,
on the second pair of leaves, when post-infection
temperatures were 20°C or higher. The older (first pair)
leaves gave susceptible reactions over a narrower range of
temperatures. Varying temperature regimes did not affect
the resistant reaction of CM90RR inoculated with race 1.

B. POST-INFECTION LIGHT EFFECTS

1. Day Length and Light Intensity

The effects of post-infection day length and light intensity upon sunflower rust were investigated in the same way as the effects of temperature. In the studies of temperature, the light factor was maintained constant at 1500 ft-c at plant level, while in these studies the temperature factor was maintained constant at $20^o \pm 1^o C$.

Sunflower plants of the varieties S37-388 and CM90RR sown January 6th, 1965 and grown in the greenhouse were selected and inoculated on February 9th, 1965. Sixteen pots variety S37-388 with 5 plants in each pot were inoculated with race 1 and 16 of variety CM90RR were inoculated with race 1 and race 3 respectively. The spore suspension contained 1.5 mg of uredospores (collected and stored on January, 29 1965) per ml.

After inoculation plants were incubated in a moist chamber for 24 hours. At the end of this period, 4 pots of each variety-race combination were transferred to each of 4 controlled environment chambers with day length and light intensities as follows:

Day length	Light intensity
16 hr	1200 ft-c
10 hr	1200 ft-c
16 hr	600 ft-c
10 hr	600 ft-c

The illumination in the chambers was provided by V.H.O. cool-white fluorescent tubes.

The effects of light factor upon (a) inoculum efficiency as determined by the density of pustules, (b) the length of the incubation period, (c) type of pustule, and (d) infectivity of teliospores, were studied.

a) Inoculum efficiency as determined by density of pustules

The number of pustules per square centimeter of leaf surface was determined on several randomly distributed areas of every leaf. The averages of the density readings from each set of 4 pots (24 plants) are shown in Table 8.

Statistical analyses of the data are shown in Appendix Tables XIII and XIV. Differences were not significant.

b) Length of incubation period

The effect of the day length and light intensity upon the length in days of the incubation period is shown as follows:

	S37-388		CM90RR	
	Race 1	Race 3	Race 1	Race 3
1200 ft-c 16 hr	5	5	5	5
1200 ft-c 10 hr	5.5-6	5.5-6	5.5-6	5.5-6
600 ft-c 16 hr	5	5	5	5
600 ft-c 10 hr	6	6	6	6

TABLE 8. Effect of day length and light intensity upon numbers of rust pustules per cm^2 of leaf surface (No.) and host reaction type (Type) of 2 sunflower varieties.

Variety	Environment	Race 1				Race 3			
		1st leaves		2nd leaves		1st leaves		2nd leaves	
		No.[1]	Type	No.	Type	No.	Type	No.	Type
CM90RR	1200 ft-c. 16 hr	0	0;	0	0;	22	3,4	21.1	3, 4
	1200 ft-c. 10 hr	0	0;	0	0;	26.2	2^+, 3^-	20.5	3^-, 3
	600 ft-c. 16 hr	0	0;	0	0;	19.4	3	20.9	3
	600 ft-c. 10 hr	0	0;	0	0;	19.4	2, 2^+	20	2^+
S37-388	1200 ft-c. 16 hr	26.1	3, 4	22.1	3, 4				
	1200 ft-c. 10 hr	23.1	2^+, 3^-	24.6	2^+, 3^-				
	600 ft-c. 16 hr	20	3	21.9	3				
	600 ft-c. 10 hr	16.6	2^+	21.4	2^+				

1) Average readings for the leaves of 24 plants (4 pots, 6 plants per pot).

It can be seen that day length exerts a slight effect on the incubation period of both races on either variety. However, a light intensity seems to have little or no effect.

c) Type of pustule

Data concerning the effect of the day length and light intensity upon the type of pustule are given in Table 8. The reaction type of race 1 on S37-388 and race 3 on CM90RR is highest under 1200 ft-c light intensity and 16 hr day length, and lowest under 600 ft-c and 10 hr day length. The difference due to the effect of day length is larger than due to the effect of light intensity.

d) Infectivity of teliospores

The procedures of the storage and inoculation of teliospores were the same as described in Experiment 1, except that teliospores were collected on February 11 and used in inoculations on March 12, 1965.

The number of pycnia on all plants was recorded 18 days after inoculation, as shown in Table 9. Most numerous infections were induced by teliospores of race 3 on CM90RR produced under 1200 ft-c, and fewer under 600 ft-c. Day length had little apparent effect. Teliospores of race 1 on S37-388 gave rise to relatively few pycnial infections. There was little apparent effect of day length or light intensity, except that spores produced under 1200 ft-c and 16 hr day length induced fewest pycnial infections. No experiment was done with race 3 on S37-388.

TABLE 9.   The effect of day length and light

intensity at which teliospores were

formed on their infectivity.

Environment	Number of pycnia 18 days after inoculation		
	S37-388	CM90RR	
	Race 1	Race 1	Race 3
1200 ft-c 16 hr	5	0	106
1200 ft-c 10 hr	26	0	132
600 ft-c 16 hr	16	0	65
600 ft-c 10 hr	21	0	54

2. Day Length

Further experiments were designed to study the possible effect of day length on sunflower rust in greater detail. On April 5, 1965, 12 pots of S37-388 and 24 pots of CM90RR were selected for uniformity of plants, from sunflower plants which had been sown on March 9 in 4-inch pots and grown in the greenhouse. The second pair of leaves was fully expanded. There were 5 plants in each pot.

Twelve pots of each variety were inoculated with race 1 and the remaining 12 pots of CM90RR with race 3. The inoculum contained 1 to 2 mg of spores (collected and stored on March 12, 1965) per ml. After inoculation in a moist chamber, 3 pots of each variety-race combination were transferred to each of 4 controlled environment chambers. The illumination in the chambers was provided by V.H.O. cool white fluorescent tubes giving 1500 ft-c at plant level. The photoperiods were 24, 16, 12, and 6 hours respectively.

The plants were examined daily to appraise (a) inoculum efficiency as determined by density of pustules, (b) length of incubation period, and (c) type of pustule. Germinability of uredospores was also determined.

a) Inoculum efficiency as determined by density of pustules

The density of pustules per square centimeter of leaf surface was determined. The average density readings from each set of 3 pots (15 plants) are shown in Table 10.

TABLE 10. Effect of day length upon numbers of rust pustules per cm^2 of leaf surface (No.) and host reaction type (Type) of 2 sunflower varieties.

| Variety | Day Length | Race 1 | | | | Race 3 | | | |
| | | 1st leaves | | 2nd leaves | | 1st leaves | | 2nd leaves | |
		No.[1]	Type	No.	Type	No.	Type	No.	Type
CM90RR	24 hr	7.0	2^-, 2	1.1	1^+, 2^-	20.6	3^- to 4	9.8	3^- to 4
	18 hr	2.5	1^+, 2^-	0.1	1^+, 2^-	15.9	3^- to 4	7.9	3^- to 4
	12 hr	3.4	1^+, 2^-	0.1	1^+, 2^-	25.9	3^- to 4	13.5	3^- to 4
	6 hr	2.0	1^+, 2^-	0.2	1^+, 2^-	26.5	3^- to 4	10.3	2^+, 3
S37-388	24 hr	24.3	3^- to 4	16.0	3^- to 4				
	18 hr	20.2	3^- to 4	16.9	3^- to 4				
	12 hr	14.6	3^- to 4	12.8	3^- to 4				
	6 hr	13.1	2^+ to 4	9.6	2^+ to 4				

1) Average readings for the leaves of 15 plants (3 pots, 5 plants per pot).

The statistical analyses are shown in Appendix Tables
XV, XVI, and XVII. The effect of day length was highly signif-
icant, but the effect of age of leaves on density of rust
pustules was even more pronounced. The first pair of leaves
showed a significantly greater density of uredial pustules
than did the second pair, under all the photoperiods. The inter-
action between age of leaves and photoperiods was not
significant. When the data were analyzed by Duncan's multiple
range test, pustule density of race 1 on S37-388 was found to
be reduced by decreases in day length. Density of race 3 on
CM90RR was apparently greater under shorter day lengths, but
the pattern of response was not consistent for the first and
second pairs of leaves.

b) Length of incubation period

Day length had no effect upon the length of the
incubation period. It took 6 days for flecks to make their
appearance in all cases. It was further observed that uredo-
spores began developing 1 day after flecking appeared under all
the different sets of conditions. However, under the 6-hr
photoperiod sporulation was observed to be less abundant than
under the other periods, which seemed to be about equal in
their effect on sporulation.

c) Type of pustules

The host reaction types under the different photoperiods
are given in Table 10. There was little evidence of photo-
periodic effects. The very abnormal photoperiods of 24 and 6
hours induced minor differences in reaction types in some race-

variety combinations from those produced under the other photo-periods. The reaction types 1 and 2 induced by race 1 on CM90RR in this experiment were unusual; in most other experiments only "0" and "0;" reactions were observed. This experiment differed from many others also in the intensity of chlorosis around the rust pustules. Chlorosis was least pronounced on leaves in the 6 hour photoperiod, and progressive-ly more intense with increasing length of day (Figs. 15 and 16).

d) Uredospore germinability

Uredospores produced by race 1 on S37-388 and by race 3 on CM90RR under 24, 18, 12 and 6 hours of illumination were collected 15 days after inoculation. The germinability of the freshly harvested spores and of spores stored at $-16^{\circ}C$ for 27 days, was determined.

The percentage germination of the freshly harvested spores is given in Appendix Tables XVIII and XIX. The statistical analyses of the data, as shown in Table 11 indicate that the fresh spores of race 1 produced at 18 and 12 hour day lengths gave higher germination values than did the spores produced under 6 and 24 hour day lengths. Those produced under the 24 hour photoperiod gave the lowest germination. Fresh spores produced by race 3 under 18 hours of light showed the highest percentage germination. The spores produced under 6, 24, and 12 hours of light gave progressively lower values. Some drying of the agar film or insufficient thickness of the
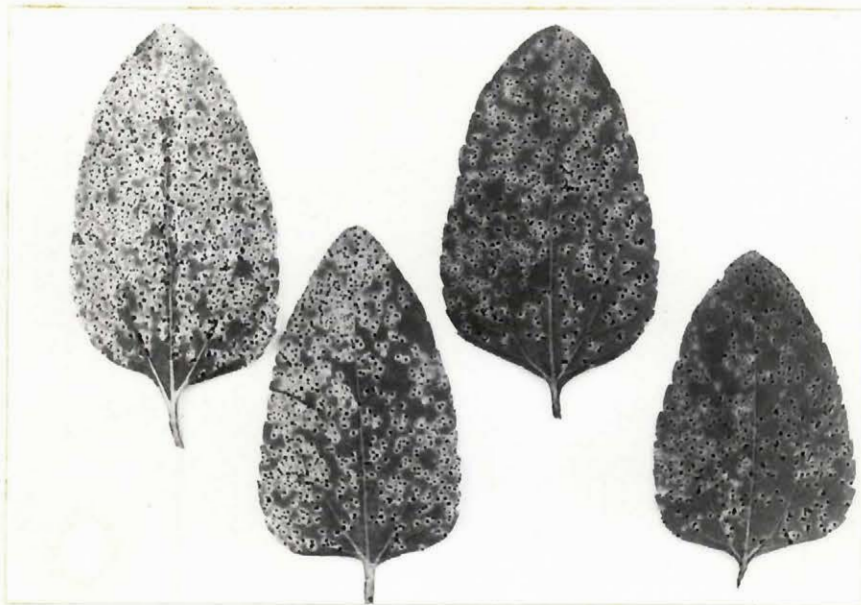
Fig. 15. Effect of day length on second pair of leaves of S37-388 inoculated with race 1. From left to right 24, 18, 12 and 6 hr, respectively.

Fig. 16. Effect of day length on second pair of leaves of CM90RR inoculated with race 3. From left to right 24, 18, 12 and 6 hr, respectively.

TABLE 11. Duncan's new multiple-range test for the effect of
photoperiods under which sunflower rust spores
were produced upon the percentage germinability of
uredospores.

Freshly Collected Spores

Race 1 on Variety S37-388

Day length	18 hr	12 hr	6 hr	24 hr
Average percentage[1] germinability	<u>87.2</u>	<u>85.8</u>	71.0	60.5

Race 3 on Variety CM90RR

Day length	18 hr	6 hr	24 hr	12 hr
Average percentage[1] germinability	87.0	72.2	59.0	32.8

Spores Stored 27 days at -16°C

Race 1 on Variety S37-388

Day length	6 hr	24 hr	12 hr	18 hr
Average percentage[1] germinability	46.1	36.3	<u>21.3</u>	<u>17.3</u>

Race 3 on Variety CM90RR

Day length	6 hr	12 hr	24 hr	18 hr
Average percentage[1] germinability	18.5	<u>6.8</u>	<u>1</u>	0.3

1) Any two means not underscored by the same line are
significantly different. Any two means underscored by
the same line are not significantly different.

agar layer on which the 12-hr spores were germinating during the test may have reduced their germination.

The percentage germination of the stored spores is given in Appendix Tables XVIII and XIX. Storage conditions reduced markedly the germinability of both races, particularly race 3. From the statistical analyses of the data presented in Table 11 it can be seen that spores of both races produced under a 6-hr day gave the highest percentage of germination. Spores of race 1 produced under 12 and 18 hours of light gave the lowest values, and those produced under 12-hr day length germinated slightly better than those produced under a 24-hr day.

3. Light Intensity

The effects of light intensities upon (a) inoculum efficiency as determined by the density of pustules, (b) the length of the incubation period, (c) type of pustule, and (d) uredospore germinability, were studied.

Sunflower plants of the varieties S37-388 and CM90RR sown April 20th, 1965 and grown in the greenhouse were selected and inoculated on May 21st, 1965. Twelve pots of each variety were inoculated with a suspension of race 1 containing approximately 1.5 mg of uredospores (collected and stored on April 19, 1965) per ml, and 12 pots of each variety with race 3.

After incubation for 24 hours in a dark moist chamber in a room kept at $23^\circ C$, 3 pots of each variety-race combination were transferred to each of 4 controlled environment chambers. Each chamber was set to give a constant temperature of $20^\circ \pm 1^\circ C$ and a day length of 16 hours. V.H.O. cool white fluorescent tubes were used to give light intensities at plant level of 500, 1200, 2000, and 2800 ft-c respectively, in the four chambers.

a) Inoculum efficiency as determined by density of pustules

The number of pustules of half of each leaf surface was counted 15 days after inoculation. The average readings for each set of 4 plants are given in Table 12.

TABLE 12.   Effect of light intensities on density of rust pustules per half leaf (No.) and on host reaction type (Type) of two sunflower varieties.

Variety	Light Intensity ft-c	Race 1				Race 3			
		1st leaves		2nd leaves		1st leaves		2nd leaves	
		No.[1]	Type	No.	Type	No.	Type	No.	Type
S37-388	2800	17.2	2^+ to 4	17.5	2^+ to 4	5.9	2^+ to 4	1.5	2^+ to 4
	2000	56.5	2^+ to 4	23.1	2^+ to 4	41.9	2^+ to 4	16.2	2^+ to 4
	1200	57	2^+ to 4	31.5	2^+ to 4	84.9	2^+ to 4	34.1	2^+ to 4
	500	31	2 to 4	12	2^+ to 4	25.1	2 to 4	9	2^+ to 4
CM90RR	2800	0	0;	0	0;	90, (5)[2]	0; 2^+ to 4	46, (30)	0; 2^+ to 4
	2000	0	0;	0	0;	98.1	0; 2^+ to 4	60, (25)	0; 2^+ to 4
	1200	0	0;	0	0;	64.5	2 to 4	16, (8)	0; 2^+ to 4
	500	0	0;	0	0;	71.0	2 to 3	58.8	0; 2^+ to 3

1) Average readings for the leaves of 12 plants (3 pots, 4 plants per pot).

2) Number inside the bracket indicates the number of flecks.

The analyses of variance of the data are given in Appendix Tables XXII, XXIII, and XXIV. Differences due to light intensity were not significant. In all combinations, the interactions between age of leaves and light intensities showed highly significant differences (Appendix Table XXV). When the data for race 1 on S37-388 were subjected to Duncan's test (Appendix Tables XXII-a and XXII-b), it was found that for the first pair of leaves light intensities of 1200 and 2000 ft-c were most favorable for rust development, while 2800 ft-c was least favorable. However, for the second pair of leaves light intensity of 1200 ft-c was optimal and 500 and 2800 ft-c were least favorable (Fig. 17). On S37-388 inoculated with race 3, on both the first and second pairs of leaves, the light intensity of 1200 ft-c was found to be the most favorable for rust development, while 2800 ft-c was least favorable, as shown in Figs. 18 and 19 as well as in Appendix Tables XXIII-a and XXIII-b. It was observed that leaves incubated under 500 ft-c were much darker that the others, and that they showed least chlorosis around the pustules.

Different results were obtained on variety CM9ORR inoculated with race 3 (Appendix Tables XXIV-a and XXIV-b). For the first pair of leaves, light intensities of 2000 and 2800 ft-c were best for rust development and light intensities of 500 and 1200 ft-c were unfavorable. However, for the second pair of leaves, light intensities of 2000 and 500 ft-c were

Fig. 17.   Effect of light intensity on second
            pair of leaves of S37-388 inoculated
            with race 1.   From left to right 500,
            1200, 2000 and 2800 ft-c respectively.

Fig. 18. Effect of light intensity on first pair
of leaves of S37-388 inoculated with
race 3. From left to right 500, 1200,
2000 and 2800 ft-c respectively.
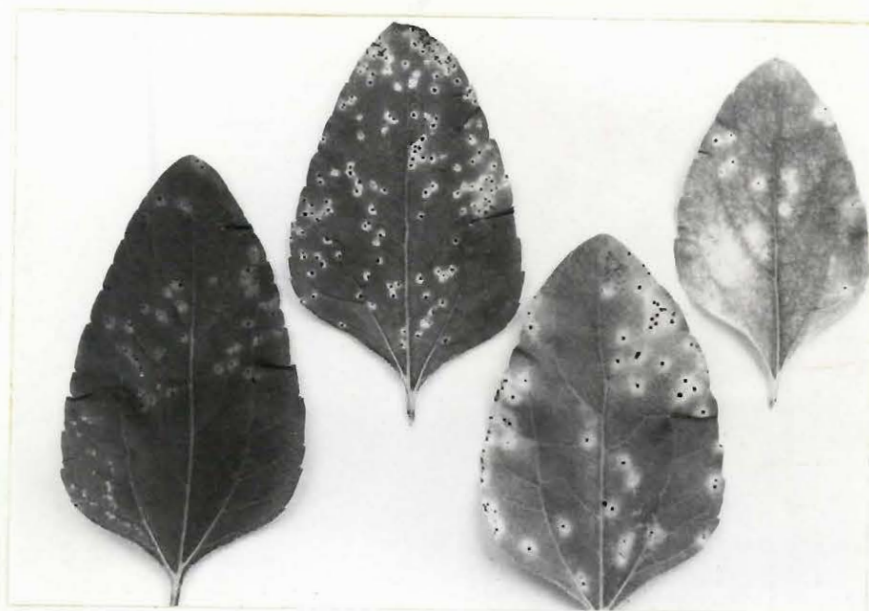
Fig. 19. Effect of light intensity on second
pair of leaves of S37-388 inoculated
with race 3. From left to right 500,
1200, 2000 and 2800 ft-c respectively.

the most favorable, and 1200 ft-c was the least favorable (Figs. 20 and 21). There was least chlorosis on leaves incubated at 500 ft-c.

b) Incubation period

The time required for the appearance of symptoms tended to be 6 days under all light conditions. Light intensity apparently did not influence the length of incubation period.

c) Type of pustule

Data concerning the effect of the light intensities upon the type of pustule are given in Table 12.

The reaction type on the first pair of leaves of S37-388 inoculated with either race was slightly higher at 1200, 2000, and 2800 ft-c than at 500 ft-c. On CM90RR inoculated with race 3 numerous flecks appeared on the younger second leaves at 1200, 2000, and 2800 ft-c, and a few on the first leaves at 2800 ft-c. The flecks were more numerous at the higher light intensities. The reaction type on older leaves tended to be slightly higher (more susceptible) under the higher light intensities than under lower ones.

Variations in light intensity did not affect the resistant (0;) reaction of CM90RR to race 1.

Under all conditions, rust developed better on the lower leaf surface than on the upper one, though the difference was not so obvious under 500 ft-c as under the three other conditions of illumination.
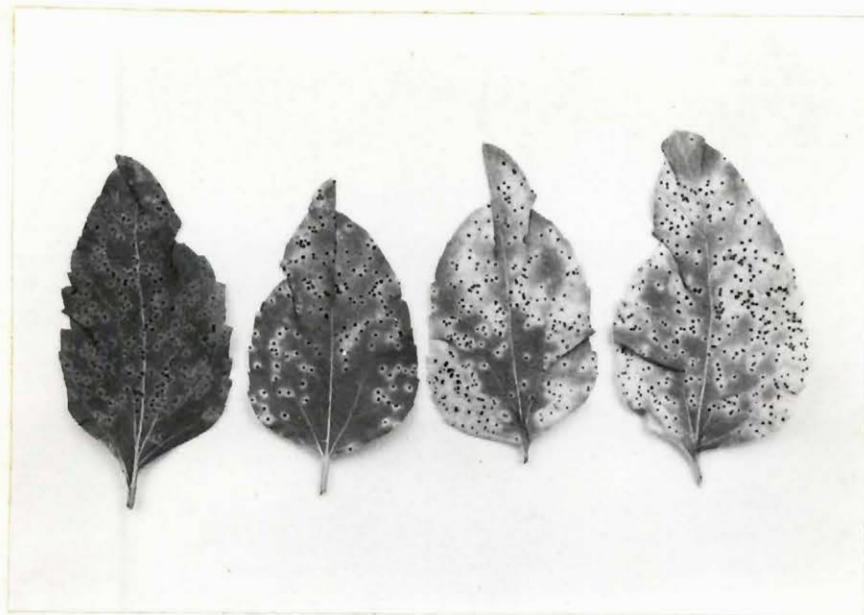
Fig. 20. Effect of light intensity on first
pair of leaves of CM90RR inoculated
with race 3. From left to right
500, 1200, 2000 and 2800 ft-c res-
pectively.
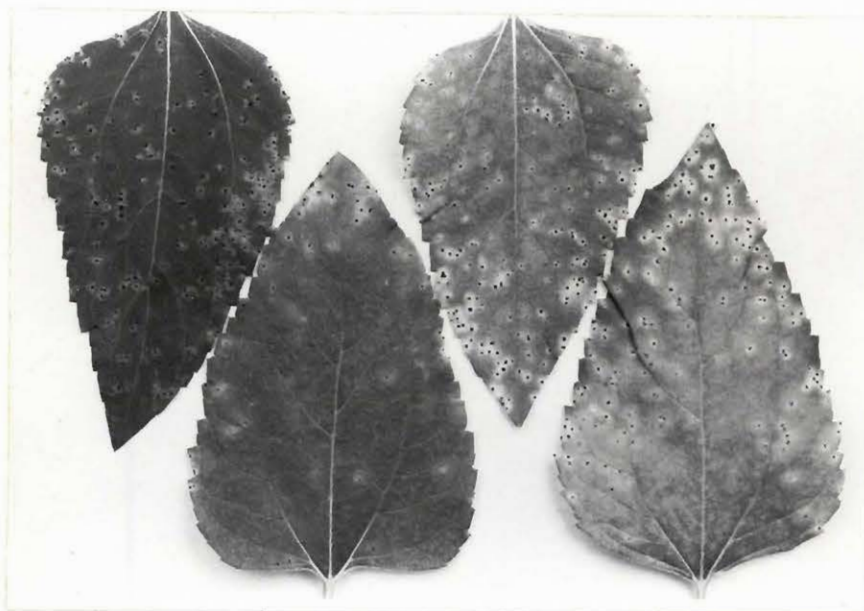
Fig. 21. Effect of light intensity on second
pair of leaves of CM90RR inoculated
with race 3. From left to right 500,
1200, 2000 and 2800 ft-c respectively.

d) <u>Uredospore germinability</u>

Spores were collected and tested for their germinability on June 9th, 1965. The germinability of only freshly collected spores was determined. As in the previous experiments the spores used in this study were race 1 produced on variety S37-388 and race 3 on CM90RR.

Percentage germination is given in Appendix Tables XXVI and XXVII. When these data were subjected to Duncan's new multiple-range test it was found, as shown in Table 13, that spores of race 1 produced under 2000 ft-c gave the highest germination while those produced under 2800 ft-c gave the lowest. On the other hand, the light intensity had no significant effect on the germinability of spores of race 3, which was much lower than that of race 1.

TABLE 13. Duncan's new multiple-range test for the
effect of light intensities under which
uredospores were produced upon the per-
centage germination of fresh uredospores.

Race 1 on Variety S37-388

Light intensities	2000 ft-c	1200 ft-c	500 ft-c	2800 ft-c
Average of percentage[1] germination	86.6	77.2	70.8	53.0

Race 3 on Variety CM90RR

Light intensities	500 ft-c	1200 ft-c	2800 ft-c	2000 ft-c
Average of percentage[1] germination	21.6	20.2	15.3	15.1

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1) Any two means not underscored by the same line are
significantly different. Any two means underscored
by the same line are not significantly different.

V.   DISCUSSION   AND   CONCLUSIONS

Preliminary experiments in the series on which this thesis is based indicated a broad range of environmental conditions which should be investigated for their effects on biology of the sunflower rust pathogen, and on the development of rust on sunflowers.   Because of some unforeseen difficulties, time did not permit repetition of critical experiments.   The discussion and conclusions which follow are therefore based on limited experimental evidence, and may need to be revised when the results of additional experiments are available.

The density of pustules on inoculated leaves was affected by the constant post-infection temperatures to which the plants were exposed.   The influence of temperature was dependent on the host variety, the rust race, and on the age of the leaves inoculated.   Race 3 on S37-388, the "universal suscept" gave essentially the same results at the temperatures tested both on the first and second pair of leaves.   On CM90RR, resistant to race 1 but susceptible to 3, race 3 gave highest pustule densities at 20° and 25 C respectively, both on the first and second leaves.   Race 1 on S37-388 gave highest pustule densities at $15^{\circ}C$, slightly lower density at 20° and $25^{\circ}C$, and the lowest density at $29^{\circ}C$ on the first leaves.   On the second pair of leaves, density was highest at 20° and $25^{\circ}C$, and much lower at 15° and $29^{\circ}C$.   This sort of variation in reactions is difficult to explain.   It may be wisest to attribute it to

unobserved variations in inoculation technique, until the exper-
iments are repeated several times with much larger numbers of
plants and the inoculation process mechanized and carefully
regulated to reduce the possibility of chance variations.

Diurnal fluctuations in post-infection temperatures, with
higher temperature during the day than during the night, had
relatively little effect on pustule density of race 1 on second
leaves of S37-388, but did influence density on the first
leaves. Highest densities were obtained on plants exposed to
$25^\circ C$ both day and night, and to those given a range of daytime
temperatures from 28° to 33° to $28^\circ C$, with $25^\circ C$ at night. Day-
time temperatures of 25° and night-time $18^\circ C$ gave somewhat
lower pustule density, and daytime temperature 33° and night
temperature $23^\circ C$ gave the lowest density. It is possible that
in the first case $18^\circ C$ at night slowed down either host or rust
development, or both, because results were better in both
regimes involving $25^\circ C$ night temperature. Presumably 16 hours
exposure to $33^\circ C$ during the day had an unfavourable effect
greater than the advantage of 23° (very close to the apparently
favourable $25^\circ C$) night temperature. Exposure to $33^\circ C$ for eight
hours in the variable daytime temperature regime apparently had
no deleterious effect.

Race 3 on both S37-388 and CM90RR gave the highest
pustule density on plants exposed to 25° during the day and
$18^\circ C$ during the night, although the values were not very much
higher than those for plants exposed to $25^\circ C$ during the night.
It will require more detailed study to determine whether it

is day temperature or night temperature which is critical for rust development, as well as to define more precisely the temperature regimes which give optimum development.

Experiments on the pre-conditioning effect of pre-inoculation temperatures were too limited to be conclusive. Pre-conditioning may well be significant in rust development, and should be investigated more fully.

Statistically significant interactions between age of leaves and temperatures as they affect pustule density are difficult to explain. The younger leaves of S37-388 appeared to be more sensitive than older leaves to the influence of temperature when inoculated with race 1. The same effect was observed on CM90RR inoculated with race 3. It was not observed, however, on S37-388 inoculated with race 3. It is important that similar studies be carried on to determine if the differences observed are obtained consistently. If they are, it may indicate that the susceptible reaction of a given combination of host variety and rust race may depend on different inter-relationships in each case.

The effect of two light intensities at each of two day lengths was studied in one experiment. In another, four different day lengths were studied at one light intensity (1200 ft-c) and four different light intensities at one day length (16 hours). In the first experiment, there was very little apparent influence of variation in day length and light intensity on development of race 3 on CM90RR, either on first

or second leaves. Decreasing light intensities and day lengths apparently resulted in decreasing intensity of infection by race 1 on first leaves of S37-388, but had little effect on the younger second leaves. Decreasing day lengths under constant light intensity apparently increased pustule density of race 3 under CM90RR on the first leaves, but had relatively little effect on second leaves. Pustule density of race 1 on S37-388 decreased with decreasing day length, both on first and second leaves.

Density of rust pustules of race 3 on CM90RR was greater on plants exposed to 2800 and 2000 ft-c than at 1200 or 500 ft-c on first leaves, but the pattern was erratic on second leaves. Pustule density of race 3 on S37-388 was highest at 1200 ft-c, lower at 2000, and lowest at 2800 and 500 ft-c on both first and second leaves. Race 1 on S37-388 gave highest densities at 1200 and 2000 ft-c, and lower densities at 2800 and 500 ft-c, both on first and second leaves.

Sunflower rust will develop quite well at light intensities from 1200 - 2000 ft-c and day lengths of 12-18 hours. In order to discover optimum conditions, however, it would be necessary to vary day lengths and light intensity simultaneously in a greater number of combinations than was possible in these experiments, with a definite possibility of finding different optima for various host-race combinations.

It has been observed by numerous investigators working with cereal rusts particularly, but also with sunflower rust,

that the best infections and clearest reaction types occur under greenhouse conditions from late February to early April. It was hoped that these experiments would indicate if temperature, day length, or light intensity were the critical factors responsible for good results during the spring. No clear-cut answers have yet been obtained. It is possible that the apparently ideal spring-time conditions in the greenhouse can be duplicated and analysed only by programming diurnal variations in each of these three factors simultaneously.

In the experiment on the effects of light intensity, many flecks were observed on leaves of CM90RR inoculated with race 3 and incubated at 2800 and 2000 ft-c, particularly on the younger second leaves. It is possible that the high light intensity interfered with normal development of some of the rust infections. This does not seem very probable, however, as the number of well-developed uredial pustules was as high or higher at 2800 and 2000 ft-c than at 1200 or 500 ft-c.

The incubation period, defined as the length of time between inoculation and the appearance of earliest symptoms or flecking, did not vary greatly with changes in the environmental conditions studied, within the ranges used. Other criteria appeared to be more important in determining the influence of environment on sunflower rust.

Reaction type, although it is expressed in numerals, is essentially a qualitative indication of the inter-action of host and rust pathogens. Cereal rust investigators have long

known that the definitely resistant and definitely susceptible reaction types are relatively stable and are not usually affected markedly by minor changes in environmental conditions. Intermediate reaction types, such as moderately susceptible or moderately resistant, however, can more readily be changed by changing environmental conditions. In some host-pathogen combinations, exceeding a critical temperature may change a highly resistant to a highly susceptible reaction. If plants are exposed to extreme temperature conditions, even highly susceptible varieties may fail to show symptoms of infection.

When planning the experiments reported above, it was not thought worth while to include environmental extremes beyond the limits likely to be encountered by sunflower plants either in the field or under usual greenhouse conditions. Within the range of temperatures studied, no dramatic changes in reaction type were observed. A temperature of $33^{\circ}C$ continuing throughout a 16-hour day was unfavourable for rust development, resulting in moderately resistant reaction types in host-pathogen combinations that appeared susceptible under more favourable conditions. The resistance of CM90RR to race 1 was not reduced by any of the conditions studied. Variations in day length and light intensity had relatively little effect on reaction type in any of the variety-race combinations.

Germinability of fresh spores of race 1 was not influenced by the temperature at which they were produced. Effects of temperature under which the spores were produced on

germinability of fresh uredospores of race 3 were statistically significant, but as the germination ranged only from 99 - 89%, the effect was not very striking. The temperature under which uredospores were produced apparently had much greater effect on their longevity in storage in the deep freezer. It has been reported (Sackston, 1960) that uredospores of P. helianthi survived storage at -10° to -22°C for five years with no apparent loss of germinability or ability to infect plants. In the experiments reported here, storage at -16°C for only 60 days reduced germinability very markedly. Spores of race 1 produced at 25° and 20°C retained significantly higher germinability than those produced at 15° and 29°C. Spores of race 3 produced at 15° appeared more resistant to storage than those produced at higher temperatures. It is not known how the temperature at which the spores are produced affects their longevity in storage. It is quite probable that spores produced in the greenhouse or in controlled environment cabinets may be much less resistant to storage than the field-produced spores used in the earlier work.

Teliospores are essential for the over-wintering of the rust organism and for the initiation of new infections in the spring for an autoecious rust such as P. helianthi, which is dependent on local inoculum for survival in most areas. In order to make genetic studies on the rust pathogen, it is essential to be able to produce teliospores under experimental conditions, and to be able to induce them to germinate and infect test plants.

The conditions which induce telial formation and affect the germinability and infectivity of the teliospores are not well understood. It has been suggested that environmental factors may influence the metabolism of the host and thus initiate the formation of telia rather than uredia by the pathogen (Waters, 1928).

Teliospores of both races formed earlier on sunflower plants kept at 15° and 20°C than at 25° or 29°C. Teliospores were also more abundant at the lower temperatures. Teliospores formed at 15°C germinated readily after storage for about a month and induced numerous pycnial infections on test plants. Teliospores formed at 20°C induced relatively few pycnial infections. Those produced at 25° and 29°C failed to induce any pycnial infections. Although no previous work has been reported with teliospores of P. helianthi, Johnson (1931) found that the temperature under which teliospores of wheat stem rust are formed affects their germinability.

The effect on germinability of the day length and light intensity under which teliospores were produced was studied. The plants were grown at a constant temperature of 20°C, which although not optimum was satisfactory for teliospore production. Teliospores of race 3 produced on CM90RR at 1200 ft-c and either 16 or 10 hours day-length gave rise to approximately twice as many pycnial infections as those

produced at 600 ft-c and either 16 hours or 10 hours day-length.
Teliospores of race 1 on S37-388 produced relatively few pycnial
infections. There was very little apparent effect of day length
or light intensity under which the teliospores were produced on
their subsequent infectivity.

Unfortunately, it was not possible to compare the
effects of specific environmental conditions on germinability
of teliospores and of uredospores, as samples for the various
germinability tests were taken from different experiments.
Teliospore formation and infectivity seem to be favoured by
temperatures slightly below those which are optimum for
uredospore production. Temperatures which were too high for
best uredial production inhibited germination of teliospores
completely.

Observations in other experiments have indicated that
teliospores formed in early spring in the greenhouse may
germinate better than those formed at other times. It will be
necessary to repeat experiments with more combinations of
temperature, light intensity, and day length, to determine
conditions which are optimum both for teliospore formation and
for their subsequent germinability.

As indicated earlier, limitations of time prevented
desirable repetition and amplification of these experiments.
The results obtained indicate that temperature within the
range studied has a greater effect on rust development than
either day length or light intensity.

VI. SUMMARY

The effect of various temperatures, light intensities, and day length on the development on sunflower rust in controlled environment cabinets was measured by determining density of pustules on inoculated leaves, reaction types, germinability of freshly harvested uredospores and of spores stored in a deep-freezer, production of teliospores, and infectivity of stored teliospores.

The rust races used were 1 and 3. The sunflower varieties studied were S37-388, susceptible to both rust races, and CM9ORR, resistant to race 1 and susceptible to race 3.

The effect of constant post-infection temperatures of 15°, 20°, 25°, and $29^{o}C$ on pustule density varied with rust race, host variety, and age of leaves inoculated (first or second pair of leaves). Density tended to be higher at $25^{o}C$ than at the other temperatures. Differences in reaction type were slight. Percentage germination of fresh spores of race 3 was highest for spores produced at $20^{o}C$; no differences were obvious among the other samples. Percentage germination of stored spores was highest for race 1 produced at 20° and $25^{o}C$, and for race 3 at $15^{o}C$.

Race 1 formed teliospores sooner than did race 3, and more abundantly at 15° and $20^{o}C$ than at 25° and $29^{o}C$. Teliospores produced at 15 C gave numerous pycnial infections, those produced at $20^{o}C$ fewer, and those at 25° and $29^{o}C$, none.

Effects of varying day-night post-infection temperatures were studied with constant day length and light intensity using the following regimes: Day temperature (D) 25°C, night temperature (N) 25°C; D-25°, N-18°; D-33°, N-23°; and D changing from 28° to 33° to 28°, N-25°C. The effect of various temperature regimes on pustule density varied with host rust race, host variety, and age of leaves. Lowest pustule density resulted from exposure to day temperature of 33° and night temperature of 23°C. Reaction types also were lowest on plants under these conditions. Race 1 appeared to give best results when night temperature was 25°C, and race 3, 18°C.

Differences in pustule density and infection type attributable to varying the pre-inoculation and post-inoculation temperatures were not statistically significant. Interactions between temperature and leaf age were significant in some cases.

The effects of day lengths of 24, 18, 12, and 6 hours respectively varied with rust race and host variety. Pustule density of race 1 on S37-388 was reduced by decreasing day length, but race 3 on CM90RR was apparently higher under shorter days. Fresh spores germinated best if produced under 18 or 12 hour days.

Effects of light intensities of 2800, 2000, 1200, and 500 foot candles respectively on pustule density were not significant. There were highly significant differences attributable to interactions between light intensity and age of leaves. On S37-388 results tended to be best at 1200 or 2000 ft-c, on CM90RR at 2000 ft-c.

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Note on Appendix Tables

In the analyses of variance, the significance of the "f" values for the interactions was determined in each analysis. Where the interactions were significant, the interaction mean squares were used to calculate "f" values for the main treatments. Where the interactions were not significant, the error mean squares were used to calculate "f" values for the main treatments.

VIII.   APPENDIX TABLES

APPENDIX TABLE 1.   Analysis of variance for the data
of the effect of post-infection constant
temperature on the density of pustules on
variety S37-388 inoculated with race 1.

Source of variance.	DF	F	5%	1%
Age of leaves	1	3.7	10.13	34.12
Temperature	3	0.9	9.28	29.46
Age of leaves x Temperature	3	5.46**	3.24	5.29
Error	16			
Total	23			

** Significant at 1%

APPENDIX   TABLE I-a   Duncan's new multiple-range

test for the data of the first pair

of leaves.

Value of P	2	3	4
SSR	3.26	3.39	3.47
LSR	5.64	5.86	6.00

Temperature	15°C	25°C	20°C	29°C
Average of pustule density[1)	19.7	16.7	16	13.7

1) Any two means underscored by the same line are not
   significantly different. Any two means not under-
   scored by the same line are significantly different.

APPENDIX TABLE I-b Duncan's new multiple-range test for the data of the second pair of leaves.

Value of P	2	3	4
SSR	3.26	3.39	3.47
LSR	4.99	5.19	5.31

Temperature	$25^\circ C$	$20^\circ C$	$15^\circ C$	$29^\circ C$
Average of pustule density	15.7	15	7.3	7.3

APPENDIX TABLE II. Analysis of variance for the data
of the effect of post-infection constant
temperature on pustule density on variety
CM90RR inoculated with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	0.006	4.49	8.53
Temperature	3	13.9**	3.24	5.29
Age of leaves x Temperature	3	0.6	3.24	5.29
Error	16			
Total	23			

** Significant at 1%

APPENDIX TABLE II-a   Duncan's new multiple-range
test for the data of the first
pair of leaves.

Value of P	2	3	4
SSR	3.26	3.39	3.47
LSR	8.88	9.22	9.44

Temperature	$25^{\circ}C$	$20^{\circ}C$	$15^{\circ}C$	$29^{\circ}C$
Average of pustule density	21	15.7	12	9

APPENDIX TABLE II-b Duncan's new multiple-range
test for the data of the second pair
of leaves.

Value of P	2	3	4
SSR	3.26	3.39	3.47
LSR	6.32	6.58	6.73

Temperature	25°C	20°C	15°C	29°C
Average of pustule density	22.7	18.3	10.7	6.3

APPENDIX TABLE III. Analysis of variance for the data of the effect of post-infection constant temperature on pustule density on variety S37-388 inoculated with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	0.24	4.75	9.33
Temperature	2	0.22	3.89	6.93
Age of leaves x Temperature	2	0.16	3.89	6.93
Error	12			
Total	17			

APPENDIX TABLE IV. Percentage germinability of fresh uredospores of race 1 produced on variety S37-388 at four different constant temperatures.

Determinations	Temperature			
	15°C	20°C	25°C	29°C
1	100	100	97	100
2	100	98	100	100
3	100	100	100	100
4	100	100	100	98
5	98	100	100	95
6	100	100	100	100
7	100	100	97	95
8	100	100	97	100
9	100	100	100	100
10	100	100	100	100
Average	100	100	99	99

APPENDIX TABLE V. Percentage germinability of fresh uredospores of race 3 produced on variety CM90RR at three different constant temperatures.

Determination	Temperature		
	20°C	25°C	29°C
1	100	96	100
2	100	97	94
3	97	98	92
4	100	97	93
5	100	97	96
6	100	100	91
7	100	100	81
8	98	97	87
9	100	96	81
10	98	96	79
Average	99	97	89

APPENDIX TABLE VI. Percentage germinability of stored uredospores of race 1 produced on variety S37-388 at four different temperatures.

Determination	Temperature			
	15°C	20°C	25°C	29°C
1	72	83	74	68
2	70	92	60	71
3	65	73	71	59
4	52	66	85	52
5	59	71	88	64
6	56	74	83	59
7	74	71	88	58
8	45	80	79	41
9	70	88	87	47
10	67	75	90	29
Average	63	77	81	55

APPENDIX TABLE VII. Percentage germinability of stored uredospores of race 3 produced on variety CM90RR at four different temperatures.

Determinations	Temperature			
	$15^{\circ}C$	$20^{\circ}C$	$25^{\circ}C$	$29^{\circ}C$
1	35	32	25	0
2	38	33	28	3
3	54	23	26	4
4	50	13	21	2
5	25	31	33	2
6	39	26	21	2
7	30	17	24	2
8	41	21	41	5
9	31	19	9	2
10	29	24	20	4
Average	37	24	25	3

APPENDIX TABLE VIII. The analysis of variance for the data of the effect of day-night post-infection temperature on the density of pustules on variety S37-388 inoculated with race 1.

Source of variation	DF	F	5%	1%
Age of leaves	1	10.2*	10.13	34.12
Temperature	3	6.3	9.28	29.46
Age of leaves x Temperature	3	2.89*	2.76	4.13
Error	56			
Total	63			

* Significant at 5%

APPENDIX TABLE VIII-a   Duncan's new multiple-range
test for the data of the first pair
of leaves.

Value of P	2	3	4	
SSR	2.90	3.04	3.13	
LSR	12.88	13.50	13.90	

Temperature				
Day	$25^{o}C$	$25^{o}C$	$25^{o}C$	$33^{o}C$
Night	$33^{o}C$	$25^{o}C$	$18^{o}C$	$23^{o}C$
Average of pustule density	96.8	95.6	78.8	56.6

APPENDIX TABLE VIII-b Duncan's new multiple-range
test for the data of the second pair
of leaves.

Value of P	2	3	4	
SSR	2.90	3.04	3.13	
LSR	13.11	13.74	14.14	
Temperature Day	25°C	25°C	25°C	33°C
Temperature Night	33°C	25°C	18°C	23°C
Average of pustule density	73.4	67.8	64.5	53.1

APPENDIX TABLE VIII-c "T" test for the influence of leaf age on effect of day-night post-infection temperatures on the density of pustules on variety S37-388 inoculated with race 1.

Day-night Temperature $\pm$ 1°C	t value		
	obtained	Tabulated 5%	1%
25°C - 18°C	1.78	2.14	2.98
25°C - 25°C	4.86*	2.14	2.98
25°C - 33°C	5.12**	2.14	2.98
33°C - 23°C	0.54	2.14	2.98

* Significant at 5%

** Significant at 1%

APPENDIX TABLE IX. The analysis of variance of the
data of the effect of day-night post-
infection temperature on the density
of pustules on variety S37-388
inoculated with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	37.3**	4.00	7.08
Temperature	3	24.6**	2.76	4.13
Age of leaves x Temperature	3	2.52	2.76	4.13
Error	56			
Total	63			

** Significant at 1%

APPENDIX TABLE IX-a Duncan's new multiple-range test for the data of the first pair of leaves.

Value of P	2	3	4	
SSR	2.90	3.04	3.13	
LSR	13.25	13.89	14.30	
Temperature Day	25°C	25°C	25°C	33°C
Night	18°C	25°C	33°C	23°C
Average of pustule density	95.2	89.0	78.8	63.9

APPENDIX TABLE IX-b   Duncan's new multiple-range
test for the data of the second pair
of leaves.

Value of P		2	3	4
SSR		2.90	3.04	3.13
LSR		11.98	12.56	12.93

Temperature	Day	25°C	25°C	25°C	33°C
	Night	18°C	33°C	25°C	23°C
Average of pustule density		86	65.5	57.6	42.8

APPENDIX TABLE X.   The analysis of variance of the
               data of the effect of day-night post-
               infection temperature on the density
               of pustules on variety CM90RR
               inoculated with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	45.7**	10.13	34.12
Temperature	3	12.9*	9.28	29.46
Age of leaves x Temperature	3	3.28*	2.76	4.13
Error	56			
Total	63			

* Significant at 5%

** Significant at 1%

APPENDIX TABLE X-a Duncan's new multiple-range
test for the data of the first
pair of leaves.

Value of P		2	3	4	
	SSR	2.90	3.04	3.13	
	LSR	13.25	13.89	14.30	
Temperature	Day	25°C	25°C	25°C	33°C
	Night	18°C	33°C	25°C	23°C
Average of pustule density		99.8	83.4	77.9	52.8

APPENDIX TABLE X-b   Duncan's new multiple-range
test for the data of the second
pair of leaves.

Value of P	2	3	4
SSR	2.90	3.04	3.13
LSR	9.69	10.15	10.45

Temperature	Day	25°C	25°C	25°C	33°C
	Night	18°C	33°C	25°C	23°C
Average of pustule density		61.4	58.5	30.5	24.8

APPENDIX   TABLE X-c   "T" test for the influence of leaf

age on effect of day-night post-infection

temperatures on the density of pustules

on variety CM9ORR inoculated with race 3.

Day-night post-infection Temperature $\pm$ 1°C	t value		
		Tabulated	
	Obtained	5%	1%
25°C - 18°C	5.89**	2.14	2.98
25°C - 25°C	10.37**	2.14	2.98
25°C - 33°C	3.89**	2.14	2.98
33°C - 23°C	5.72**	2.14	2.98

** Significant at 1%

APPENDIX TABLE XI.   Analysis of variance for the data
of the effect of preinoculation and post-
inoculation temperature upon the density
of pustules on variety S37-388 inoculated
with race 1.

Source of variation	DF	F	5%	1%
Age of leaves	1	1.50	4.00	7.08
Temperature	6	1.32	2.25	3.12
Age of leaves x Temperature	6	1.43	2.25	3.12
Error	70			
Total	83			

APPENDIX TABLE XII. Analysis of variance for the data
of the effect of preinoculation and post-
inoculation temperatures upon the density
of pustules on variety CM90RR inoculated
with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	0.49	5.99	13.75
Temperature	6	3.28	4.28	8.47
Age of leaves x Temperature	6	5.03**	2.25	3.12
Error	70			
Total	83			

** Significant at 1%

APPENDIX   TABLE   XII-a   Duncan's new multiple-range test
for the data of the first pair of leaves.

Value of P	2	3	4	5	6	7	
SSR	2.86	3.01	3.10	3.17	3.22	3.27	
LSR	6.69	7.04	7.25	7.42	7.53	7.65	

Temperature		2	3	4	5	6	7
	Pre-	20°C	15°C	24°C	15°C	29°C	20°C	29°C
	Post-	20°C	24°C	24°C	15°C	15°C	29°C	29°C
Average of pustule density		20.7	19.8	15.2	11.3	10.5	8	2.8

APPENDIX TABLE XII-b Duncan's new multiple-range test for
the data of the second pair of leaves.

Value of P	2	3	4	5	6	7
SSR	2.86	3.01	3.10	3.17	3.22	3.27
LSR	4.98	5.24	5.39	5.52	5.60	5.69

Temperature								
Pre-	$15^{\circ}C$	$15^{\circ}C$	$20^{\circ}C$	$24^{\circ}C$	$20^{\circ}C$	$29^{\circ}C$	$29^{\circ}C$
Post-	$15^{\circ}C$	$24^{\circ}C$	$20^{\circ}C$	$24^{\circ}C$	$29^{\circ}C$	$29^{\circ}C$	$15^{\circ}C$

Average of pustule density	25.8	17.2	16.7	11.5	6.8	6.8	6.7

APPENDIX TABLE XII-c "T" test for the influence of
leaf age on effect of preinoculation
and postinoculation temperatures on
the density of pustules on variety
CM9ORR inoculated with race 3.

Pre- and Post-inoculation Temperatures $\pm$ 1°C	t value		
	Obtained	Tabulated 5%	1%
15°C - 15°C	4.63**	2.23	3.17
15°C - 24°C	0.86	2.23	3.17
20°C - 20°C	0.90	2.23	3.17
20°C - 29°C	0.53	2.23	3.17
24°C - 24°C	1.19	2.23	3.17
29°C - 15°C	1.95	2.23	3.17
29°C - 29°C	2.44*	2.23	3.17

* Significant at 5%

** Significant at 1%

APPENDIX TABLE XIII. Analysis of variance for the data of the effect of day length and light intensity upon the density of pustules on variety S37-388 inoculated with race 1.

Source of variation	DF	F	5%	1%
Age of leaves	1	0.49	10.13	34.12
Light	3	2.70	9.28	29.46
Age of leaves x Light	3	1.48	2.76	4.13
Error	56			
Total	63			

APPENDIX TABLE XIV. Analysis of variance for the data of the effect of day length and light intensity upon the density of pustules on variety CM90RR inoculated with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	0.47	10.13	34.12
Light	3	1.04	9.28	29.46
Age of leaves x Light	3	0.99	2.76	4.13
Error	56			
Total	63			

APPENDIX TABLE XV. Analysis of variance for the data of the effect of post-infection photo-periods upon the density of pustules on variety S37-388 inoculated with race 1.

Source of variation	DF	F	5%	1%
Age of leaves	1	8.69**	4.00	7.08
Day length	3	8.21**	2.76	4.13
Age of leaves x Day length	3	0.97	2.76	4.13
Error	72			
Total	79			

** Significant at 1%

APPENDIX TABLE XV-a.   Duncan's new multiple-range test

for the data of the first pair of leaves.

Value of P	2	3	4
SSR	2.86	3.01	3.10
ISR	6.84	7.19	7.41

Day length	24 hr	18hr	12hr	6hr
Average of pustule density	24.3	20.2	14.6	13.1

APPENDIX TABLE XV-b. Duncan's new multiple-range test

for the data of the second pair of leaves.

Value of P	2	3	4
SSR	2.86	3.01	3.10
LSR	4.52	4.76	4.90

Day length	18hr	24hr	12hr	6hr
Average of pustule density	16.9	16	12.8	9.6

APPENDIX TABLE XVI. Analysis of variance of the data
of the effect of post-infection photo-
periods upon the density of pustules
on variety CM9ORR inoculated with race
3.

Source of variation	DF	F	5%	1%
Age of leaves	1	99.59**	4.00	7.08
Day length	3	8.64**	2.76	4.13
Age of leaves x Day length	3	2.08	2.76	4.13
Error	72			
Total	79			

** Significant at 1%

APPENDIX   TABLE   XVI-a.   Duncan's new multiple-range test

for the data of the first pair of leaves.

Value of P	2	3	4
SSR	2.86	3.01	3.10
LSR	5.63	5.93	6.12

Day length	6hr	12hr	24hr	18hr
Average of pustule density	26.5	25.9	20.6	15.9

APPENDIX TABLE XVI-b. Duncan's new multiple-range test for the second pair of leaves.

Value of P	2	3	4
SSR	2.86	3.01	3.10
LSR	3.78	3.97	4.09

Day length	12hr	6hr	24hr	18hr
Average of pustule density	13.5	10.3	9.8	7.9

APPENDIX TABLE XVII. Analysis of variance of the data of the effect of post-infection photoperiods upon the density of pustules on variety CM90RR inoculated with race 1.

Source of variation	DF	F	5%	1%
Age of leaves	1	37.4^{**}	4.00	7.08
Day length	3	6.28^{**}	2.76	4.13
Age of leaves x Day length	3	2.72	2.76	4.13
Error	56			
Total	63			

** Significant at 1%

APPENDIX TABLE XVII-a. Duncan's new multiple-range test for the data of the first pair of leaves.

Value of P	2	3	4
SSR	2.90	3.04	3.13
LSR	3.04	3.19	3.29

Day length	24hr	12hr	18hr	6hr
Average of pustule density	7	3.4	2.5	2

APPENDIX TABLE XVII-b. Duncan's new multiple-range test
for the data of the second pair of leaves.

Value of P	2	3	4
SSR	2.90	3.04	3.13
LSR	0.78	0.82	0.84

Day length	24hr	6hr	12hr	18hr
Average of pustule density	1.12	0.25	0.12	0

APPENDIX TABLE XVIII   Percentage germinability of fresh
uredospores of race 1 produced on variety
S37-388 under four different photoperiods.

| Determinations | Day length of production | | | |
	24hr	18hr	12hr	6hr
1	68	89	84	73
2	56	85	81	71
3	61	93	80	73
4	65	88	87	62
5	55	88	86	72
6	68	80	92	75
7	63	85	88	75
8	57	89	88	71
9	59	85	87	63
10	53	90	85	75
Average	60.5	87.2	85.8	71.0

APPENDIX TABLE XIX Percentage germinability of fresh uredospores of race 3 produced on variety CM90RR under four different day lengths.

| Determinations | Day lengths during production |
	24hr	18hr	12hr	6hr
1	54	84	28	69
2	67	82	50	67
3	53	89	40	75
4	79	88	23	77
5	72	84	50	85
6	57	96	32	86
7	39	90	33	65
8	64	86	20	70
9	50	83	27	71
10	55	88	25	57
Average	59.0	87.0	32.8	72.2

APPENDIX TABLE XX Percentage germinability of stored uredospores of race 1 produced on variety S37-388 under four different day lengths.

Determinations	Day length during production			
	24hr	18hr	12hr	6hr
1	53	13	24	55
2	42	34	26	32
3	24	25	12	48
4	41	28	24	45
5	24	15	26	36
6	42	13	17	39
7	32	6	13	45
8	28	19	33	66
9	45	7	15	45
10	32	13	23	50
Average	36.3	17.3	21.3	46.1

APPENDIX TABLE XXI   Percentage germinability of stored uredospores of race 3 produced on variety CM90RR at four conditions of different day length.

Determinations	Day length of production			
	24hr	18hr	12hr	6hr
1	1	0	23	13
2	2	0	0	21
3	2	0	0	11
4	1	0	2	11
5	0	0	12	11
6	0	0	6	48
7	3	1	0	16
8	0	2	4	10
9	0	0	0	26
10	1	0	3	18
Average	1	0.3	6.8	18.5

APPENDIX TABLE XXII   Analysis of variance of the data of
the effect of light intensities upon the
density of pustules on variety S37-388 in-
oculated with race 1.

Source of variation	DF	F	5%	1%
Age of leaves	1	7.30	10.13	34.12
Light intensity	3	3.42	9.28	29.46
Age of leaves x Light intensity	3	12.3**	2.76	4.13
Error	56			
Total	63			

** Significant at 1%

APPENDIX TABLE XXII-a   Duncan's new multiple-range test
for the data of the first pair of leaves.

Value of P	2	3	4	
SSR	2.90	3.04	3.13	
LSR	8.93	9.36	9.64	

	1200 ft-c	2000 ft-c	500 ft-c	2800 ft-c
Light intensity				
Average of pustule density	57	56.5	31	17.2

APPENDIX TABLE XXII-b Duncan's multiple-range test for
the data of the second pair of leaves.

Value of P	2	3	4	
SSR	2.90	3.04	3.13	
LSR	7.77	8.14	8.39	
Light intensity	1200 ft-c	2000 ft-c	2800 ft-c	500 ft-c
Average of pustule density	31.5	23.1	17.5	12

APPENDIX   TABLE   XXIII   Analysis of variance of the data
of the effect of light intensity upon the
density of pustules on variety S37-388
inoculated with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	6.04	10.13	34.12
Light intensity	3	5.85	9.28	29.46
Age of leaves x Light intensity	3	26.8**	2.76	4.13
Error	56			
Total	63			

** Significant at 1%

APPENDIX TABLE XXIII-a Duncan's new multiple-range test
for the data of the first pair of leaves.

Value of P	2	3	4	
SSR	2.90	3.04	3.13	
LSR	9.80	10.28	10.58	
Light intensity	1200 ft-c	2000 ft-c	500 ft-c	2800 ft-c
Average of pustule density	84.9	41.9	25.1	5.9

APPENDIX TABLE XXIII-b   Duncan's new multiple-range test

for the data of the second pair of leaves.

Value of P	2	3	4
SSR	2.90	3.04	3.13
LSR	5.10	5.35	5.51

Light intensity	1200 ft-c	2000 ft-c	500 ft-c	2800 ft-c
Average of pustule density	34.1	16.2	9	1.5

APPENDIX TABLE XXIV Analysis of variance of the data of the effect of light intensities upon the density of pustules on variety CM90RR inoculated with race 3.

Source of variation	DF	F	5%	1%
Age of leaves	1	19.2*	10.13	34.12
Light intensity	3	4.1	9.28	29.46
Age of leaves x Light intensity	3	7.1**	2.76	4.13
Error	56			
Total	63			

* Significant at 5%
** Significant at 1%

APPENDIX TABLE XXIV-a Duncan's new multiple-range test
for the data of the first pair of leaves.

Value of P	2	3	4
SSR	2.90	3.04	3.13
LSR	13.48	14.14	14.55

	2000 ft-c	2800 ft-c	500 ft-c	1200 ft-c
Light intensity				
Average of pustule density	98.1	90	71	64.5

APPENDIX TABLE XXIV-b   Duncan's new multiple-range test

for the data of the second pair of leaves.

Value of P	2	3	4
SSR	2.90	3.04	3.13
LSR	11.40	11.95	12.30

	2000 ft-c	500 ft-c	2800 ft-c	1200 ft-c
Light intensity				
Average of pustule density	60.0	58.8	46.0	16

APPENDIX TABLE XXV. "T" test for the influence of leaf age on effect of light intensities on density of pustules on variety S37-388 inoculated with races 1 and 3 and CM9ORR inoculated with race 3.

Light intensities	S37-388						CM9ORR		
	Race 1			Race 3			Race 3		
	t value			t value			t value		
	Obtained	5%	1%	Obtained	5%	1%	Obtained	5%	1%
500 ft-c	4.69**	2.14	2.98	5.69**	2.14	2.98	2.35*	2.14	2.98
1200 ft-c	5.21**	"	"	10.10**	"	"	8.11**	"	"
2000 ft-c	8.07**	"	"	5.27**	"	"	6.02**	"	"
2800 ft-c	0.098	"	"	4.21**	"	"	6.48**	"	"

* Significant at 5%

** Significant at 1%

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APPENDIX TABLE XXVI. Percentage germinability of fresh uredospores of race 1 produced on variety S37-388 under four different light intensities.

Determinations	Light intensity			
	500 ft-c	1200 ft-c	2000 ft-c	2800 ft-c
1	67	92	75	42
2	90	61	84	58
3	67	76	88	50
4	61	71	92	49
5	75	77	78	29
6	72	83	97	52
7	73	76	92	70
8	65	83	91	53
9	53	74	88	68
10	85	79	81	59
Average	70.8	77.2	86.6	53.0

APPENDIX   TABLE   XXVII.   Percentage germinability of
fresh uredospores of race 3 produced
on variety CM90RR under four
different light intensities.

| | Light intensity | | | |
| | 500 ft-c | 1200 ft-c | 2000 ft-c | 2800 ft-c |
Determinations				
1	22	17	14	13
2	23	22	21	8
3	34	9	21	8
4	26	16	6	37
5	25	23	6	21
6	19	12	17	43
7	24	22	26	7
8	16	26	13	5
9	17	31	14	6
10	10	24	13	5
Average	21.6	20.2	15.1	15.3