EFFECT OF TEMPERATURE

ON SEVERAL CHARACTERISTICS

OF SUMMER RAPE (BRASSICA NAPUS L.)

by

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ABSTRACT

Two experiments were conducted to determine if seed size, oil content, iodine number of oil, and protein content of rapeseed could be modified by subjecting rape plants to various temperature - time treatments from early bolting till maturity. Results from constant temperature treatments from both experiments show that seed size and oil content of rapeseed and iodine number of rapeseed oil could be decreased, whereas protein content was increased by exposing rape plants to high temperature from early bolting till maturity. This was generally also true for variable temperature treatments.

Results from variable temperature treatments of both experiments show that characteristics of rapeseed were more susceptible to temperature treatment during terminal than during initial periods.
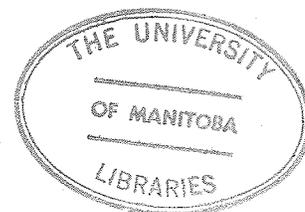
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INTRODUCTION

Fussel (8) stated that rape (_Brassica napus_ L.) has been grown in Europe since the 17th century and according to White and Bolton (20) the crop was not grown commercially for production of oil in Canada until 1942 when World War II cut off rapeseed oil imports from Oriental countries. Subsequently, advances in technologies of the food processing industries coupled with demands to fulfill requirements for edible oils of the rapidly multiplying human population of our world have spurred further interest in rapeseed production in Canada. The Commonwealth Economic Committee (3) reports that since 1955 Canada has been the world's "largest shipper of rapeseed". Thus in a short period Canada's role changed from importer to major exporter of rapeseed.

The rapid rise in the importance of the rapeseed crop in Canadian agriculture warrants an ecological study of summer rape. Knowledge about the impact of an environmental variable such as air temperature during seed development stage on the quality and quantity of seed components could be of importance in making recommendations to growers and for the integration and interpretation of results obtained from field trials. Consequently, a project was undertaken to determine the associations of several controlled temperature - time treatments with oil and protein metabolism in developing seed of summer rape.

LITERATURE REVIEW

Although studies have been made on the effect of different environments on oilseed crops such as soybeans and flax, very little has been done regarding the relationship between environment and the composition of rapeseed.

Prior to 1914 Garner et al. (9) noted from general observations that seeds produced under different conditions frequently varied in composition to such an extent that their commercial value was affected. Therefore they (9) undertook to "ascertain so far as possible the most favourable conditions for obtaining maximum yields of oil" in soybeans and cotton- seed by making studies on the effect of location, soiltype, climate, and fertilizers, in modifying oil content and seed size. The studies revealed that: 1) Differences in oil content and seed size existed between locations; 2) oil content and seed size of soybeans grown in large tile cylinders filled with the same soil type and set into the ground at different locations, varied with location and year; 3) oil oil content was reduced by the addition of nitrogen, and increased by the addition of phosphorus, but was not affected by potassium; 4) phosphorus increased seed weight, but nitrogen and potassium were ineffective in this respect. Garner et al. (9) concluded that under practical field con- ditions climate was more effective than soil type in controlling oil content and seed size, but because of the "interdependence of soil and climate" with respect to temperature and water

supply, it is difficult or impossible to develop far-reaching generalizations as to the effect of either independently of the other on plant development".

For several years after the work of Garner et al. (9), the study of environmental effects on oilseed crops by several investigators (7, 13, 14) was centered on the effects of mineral and organic fertilizers and soil type on protein content and oil content of soybeans. The studies are of particular interest because they disclosed that protein content as well as oil content can be influenced by soil fertility, and that soil treatments which increased protein content invariably decreased oil content.

Stark (15) reported in 1924 on a study dealing with the effect of soil type not only on both oil and protein contents of soybeans, but also with the effect of soil type on iodine number of soybean oil. His data (15) show that both oil and protein content of 4 varieties of soybeans differed between locations that differed in soil reaction. Acid soils were associated with high oil and low protein, whereas high lime soils were associated with low oil and high protein. Stark (15) generalized that variability in the amount of oil and protein in soybeans was probably not so much due to "geographic position or climatic conditions but rather to the plant food available and to soil reaction". However, the generalization is of limited value, firstly,

because the data are from a single year of trials only, and secondly, the paper contains no information about climatic conditions during the experiment. Furthermore, the separation of available plant food and soil pH is not entirely justified, because the availability of plant nutrients may depend on soil pH. With regard to iodine number Stark (15) found that there was considerable variation within varieties but it was not consistent enough to indicate whether location or soil type was responsible. The inconsistent data on iodine number may have been due to interaction of soil and climate.

Cartter and Hopper (2) made a 5 year study of environmental effects on seed components of 10 varieties of soybeans at 5 locations. Components studied were oil content, protein content, and iodine number of the oil. An analysis of variance of each of the 3 seed components revealed significant (.01 level) mean squares for varieties, location, years, and location x years. Significant variances for years, and location x years indicates that climate as well as soil was effectual in making up the mean squares due to location.

In another experiment Cartter and Hopper (2) observed the effect of 4 soil fertility levels on protein content, oil content, and oil iodine number of 8 varieties of soybeans during 3 seasons. Analysis of variance of protein data revealed a non-significant mean square for years, but mean mean squares for varieties, levels of soil fertility, and

varieties x years were significant. Evidently there was no
difference between year means, however the significant
varieties x years interaction indicates that some of the
means of individual varieties differed between years,
suggesting that climate can affect protein content. However,
since the variance for years was non-significant whereas the
mean square for levels of soil fertility was highly signifi-
cant it appears that soil fertility was more important than
climate in controlling protein content. For both oil content
and iodine number of oil, years and varieties each contributed
considerably more to variation than did fertility levels,
though all 3 sources of variation were statistically signifi-
cant. Therefore, it appears that climate had a greater
effect than soil fertility on oil content and iodine number
of soybean oil.

In order to determine the effect of a single climatic
factor, Weiss et al. (18) correlated some seed compositional
characters of 5 varieties of soybeans with temperatures dur-
ing the pod filling period. Temperature differentials during
the seed filling stage were controlled by planting at 4
successive 11-day intervals at each of 3 locations in three
successive years. They (18) found that for both early and
late varieties of soybeans a positive correlation existed
between oil content and mean temperature for the period of
from 50% flowering until maturity. The association of protein

and oil content was negative. Weiss et al. (18) ·found that mean temperature during seed development was negatively correlated with iodine number of soybean oil.

In an effort to discover during which stage of seed development oil production of soybeans is most sensitive to temperature differentials, Howell and Cartter (11) determined correlation coefficients between temperature and oil content of several varieties of soybeans for 10-day periods from 50 days before maturity until maturity. They (11) found that oil content and temperature were most closely correlated 20-30 days before maturity, suggesting that the influence of temperature was greatest at that time.

More recently, Howell and Cartter (12) used growth chambers to determine whether oil content of soybeans could be changed by controlling temperature during all or part of the pod filling stage. They (12) found that the oil content averaged 23.2, 20.8, and 19.5 per cent when the day temperatures of 85°, 77°, and 70° F. respectively, were used constantly during the entire pod filling stage. When the temperature was elevated to 80° F. from 70° F. for one week during the fourth, fifth, sixth, or seventh week before maturity an oil content of about 22 per cent resulted, as compared to 19.6 per cent when the temperature was elevated during the second week before maturity. These data indicate that oil content of soybeans is sensitive to temperature differentials for as short a period as one week during pod filling, and that a

critical period for oil formation exists from 4 to 7 weeks before maturity.

A study by Hopper and Johnson as summarized in Experiment Station Records (5) deals with the effect of climate on iodine number and quantity of oil in flaxseed. Their (5) data, gathered from United States Department of Agriculture reports and from commercial analysis covering the period 1911-1937, show that in North Dakota and Minnesota oil content and oil iodine number of flaxseed were negatively correlated with mean July temperature. Experiment Station Records (6) report that similar results were obtained by Dillman and Hopper who studied the effect of climate on oil content and iodine number of flaxseed. Based on the results of a 1-10 years cooperative tests at 54 experimental stations representing most of the flax growing areas in the United States, Dillman and Hopper's (6) project disclosed that in the North Central States both deficient rainfall and high July temperatures were negatively correlated with oil content and with iodine number. Crude protein content of flaxseed was negatively correlated with precipitation, and positively with July temperature.

In general, high temperature during seed development can result in depressed iodine numbers of soybean oil and of flaxseed oil. Oil content of flaxseed was depressed whereas oil content of soybeans was increased by high temperature during seed development.

MATERIALS AND METHODS

In 1959-60 two experiments were carried out at the University of Manitoba to determine whether the oil and protein contents of rapeseed and iodine number of rapeseed oil could be altered by controlling temperatures during seed development. Temperatures were maintained by use of plant growth chambers manufactured by Coldstream Refrigeration Company. Lighting in each chamber was provided by 32 General Electric FR 96T12 C.W. fluorescent lamps and 7 Westinghouse 60 watt incandescent lamps. The centre line of the gabled lamp hood was suspended 40 inches above the pots. There was no provision for the control of relative humidity.

Experiment I

Three hundred 7 inch clay pots were filled with a uniform mixture of 5 parts clay loam and 1 part sand. On May 25, 1959 these pots were seeded with Golden rape and placed in a sheltered place outdoors. The resulting seedling stand was thinned to 5 plants per pot. At the early bolting stage 150 of the pots were selected, and subsequently assigned to one of 15 groups of 10 pots each. Each pot of plants in a group was selected for the resemblance of height and number of leaves of its plants to those of a pot of plants in each of the other groups. On July 7 the material was transferred into growth chambers.

Plants were watered as needed. In general, while in

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the growth chambers this meant adding 10 ounces·of water
every 72 hours to each pot kept at 55° F., every 48 hours
to pots at 70° F. and alternate 32 and 40 hours to pots kept
at 85° F.

The photoperiod of all treatments was 13 hours.

Each group of plants was subject to a specific
temperature - time treatment. For the sake of brevity each
treatment will be described by a coded expression. Each
expression has been divided into three terms e.g. 55-70-70.
The first and the second terms each represent an 11-day
interval. The third term represents the time remaining till
maturity. The numbers represent temperatures in degrees
Fahrenheit during particular time intervals. The coded
expressions that will be used hereafter to describe treat-
ments of Exp. I are given in Table I below.

TABLE I   Coded expressions describing temperature - time
           treatments of Exp. I

55-55-55	70-70-70	85-85-85
55-55-70	70-70-55	85-85-55
55-70-70	70-55-55	85-55-55
55-55-85	70-70-85	85-85-70
55-85-85	70-85-85	85-70-70

Consideration of Table I shows that in some treatments
a temperature change was made immediately after the first

11-day interval, whereas in other treatments a temperature
change was made after the first and the second 11-day inter-
vals had lapsed. Thus a particular treatment may be described
as one that had an 11-day initial period e.g. 55-70-70, or
had a 22-day initial period e.g. 55-55-70. The third interval
will be referred to as a terminal period regardless of dura-
tion.

Data regarding 100-seed weight, oil and protein
contents of rapeseed, and refractive index of rapeseed oil
were collected from each treatment.

Experiment II

In Exp. II the type of soil, type of pot, seed lot,
number of pots seeded, method of selection, and type of
growth chambers were the same as those used in Exp. I.
However, in Exp. II five grams of fertilizer consisting of
equal parts of 16-20-0 and 0-0-60 was placed one inch below
the seed in each pot. Pots were seeded on August 14 and
placed outdoors. Subsequent seedling stands were thinned to
three plants per pot. The material was transferred to
growth chambers on October 14.

Enough water was added to keep the soil surface moist
at all times. In general this meant that plants at 70° F.
and 80° F. were watered every day and plants at 60° F. were
watered every two days. The amount of water added was not

measured, but was usually just sufficient to bring the mois-

ture level up to the water holding capacity of the soil.

The photoperiod for all treatments was 15 hours.

Each group of plants was subjected to a particular

temperature - time treatment. For the sake of brevity and

clarity each treatment will be described by a coded expression.

Each expression has been divided into three terms e.g. 80-60-80,

each term representing a period of time. The numbers describe

in degrees Fahrenheit the temperature during a specified

time period. The first or initial period represents the

time required for the plants to develop from beginning of

bolting till end of flowering. The second interval or

middle period represents a 10-day interval following end of

flowering. The third or terminal period represents the time

remaining till maturity. Obviously treatments such as

80-60-60 had only initial and terminal periods. The coded

expressions that will be used hereafter to describe treatments

are given in Table II below.

TABLE II   Coded expressions describing temperature - time
            treatments of Exp. II

60-60-60	70-70-70	80-80-80
60-70-60	70-60-70	80-60-80
60-70-70	70-60-60	80-60-60
60-80-60	70-80-70	80-70-80
60-80-80	70-80-80	80-70-70

Consideration of coded expressions in Tables I and II reveals that some treatments did not have a temperature change e.g. 55-55-55 and 60-60-60. These treatments will be referred to as constant temperature treatments. Treatments that had one or more temperature changes will be referred to as variable temperature treatments. Data from constant temperature treatments provided a means of determining the overall effects of a particular temperature on various components of rapeseed, and also provided a standard for evaluating results from variable temperature treatments. Data from variable temperature treatments provided a means of measuring the effects of a specific temperature on various seed components during a particular time period.

Data regarding 100-seed weight, oil and protein content of rapeseed, refractive index and calculated iodine number were collected from each treatment.

Statistical Techniques

Design and Analysis

The design of both experiments was an incomplete factorial. Data were analysed as in a completely randomized design. Error terms were derived from variations between determinations on samples treated alike. Differences between all treatment means pertaining to a particular seed component were tested for significance at the 5% level by Hartly's

sequential method as outlined by Snedecor (16).

Sampling

A single 100-seed weight was determined for each of the 10 pots of plants in a treatment[1]. For per cent oil content determination the rapeseed from adjacent pots in a treatment was bulked and approximately one gram of seed was analysed from each of the five bulked samples. A single refractive index reading was determined for each sample of rapeseed oil. Per cent protein determinations were made on two seed residue samples per treatment.

Laboratory Techniques

Seed Size

Pods were harvested into paper bags and subsequently stored for one week at room conditions before they were threshed. A separate bag was used for each pot of plants, thus 10 samples of seed were collected from each treatment. A single 100-seed weight was made from each sample of seed.

Oil Content Determination

The method used for determining oil content of the rapeseed was similar to that described by Comstock and Culbertson (4), with the exception of the following modifications: 1) before pressing, the seed was oven-dried at 105° C. for 15 hours; 2) a crushing pressure of 11000 pounds per square inch was applied for one minute to a

[1]   For exceptions see Appendix A and B

measured volume of approximately one gram of rapeseed; 3) seed residue, after the third decantation, was air-dried for 24 hours, and then oven-dried at 105° C. for 15 hours.

Oil content was calculated from the difference between the dry weight of the sample of whole seed and the dry weight of the seed residue left after oil extraction. Difference in weight was attributed to the oil that had been extracted.

Refractive Index and Calculation of Iodine Number of Rapeseed Oil

The solvent and oil mixture resulting from three successive decantations was air-dried for 24 hours followed by drying in a vacuum oven for 1 hour at 40° C. After cooling to room temperature a drop of oil was placed in a Bellingham and Stanley No. 465415 refractometer that had a prism temperature of 35° C. One minute was allowed for temperature equilibration of oil and prism before a reading was taken.

Refractometer readings were converted into iodine numbers utilizing the formula $12873.58 \times (n^{25} + .0036) - 18831.54 = $ I.N. as described by Anderson (1), where n^{25} equals the refractometer reading with a prism temperature of 25° C. and .0036 is a correction factor required for a prism temperature of 35° C.

Protein Content of Rapeseed Residue

Seed residue remaining after oil extraction was used for determination of protein content. Two samples of seed

residue were chosen at random from each treatment. All

residue in a sample (approximately 0.5 gm.) was analysed

for nitrogen by the Kjeldahl Method (10). Seed residue was

digested in 20 ml. concentrated H_2SO_4 in the presence of one

package of Kel-Pak no. 4 2/, for 40 minutes. After digestion

65 ml. of a solution containing 45% (wt./vol.) NaOH and 3.6%

(wt./vol.) $Na_2S_2O_3 \cdot 5H_2O$ were added. The amount of NH_3OH

was estimated by titrating with .0571 N NaOH. The protein

content was calculated by multiplying the N_2 content by the

factor 6.25.

2/   Kel-Pak is manufactured by Harshaw Scientific Co.

RESULTS AND DISCUSSION

Results and Discussion has been divided into four
main sections namely: Seed Size, Oil Content, Iodine Number,
and Protein Content, and are dealt with in that order. In
each section, results from Experiments I and II are discussed
separately but are compared in a brief summary.

Constant temperature treatments of Experiments I and
II provided a means of observing the overall effects of sev-
eral different temperatures on synthesis of various compon-
ents in rapeseed, and provided a basis for interpretation
of results from variable temperature treatments. Variable
temperature treatments provided a means for observing the
effects of increased and decreased temperature on rapeseed
components during initial, middle (Exp. II only), and
terminal periods of treatments.

Seed Size

Rapeseed size expressed as grams per 100 seeds is a measure of the total amount of material anabolized into the seed. It follows that a variation in seed size associated with a change in level of an environmental factor such as temperature could imply that elaboration of one or more seed constituents was affected by temperature. Data in Tables III and IV show that the range of Experiment I seed sizes measured in grams per 100 seeds was 0.2786, and in Experiment II the range was 0.1219. The relatively large ranges indicate that temperature - time treatments affected the seed size of rapeseed.

Experiment I

Constant Temperature Treatments

The strongly negative association between seed size and temperature when a uniform temperature was maintained from bolting to maturity is illustrated by data from Table III where comparisons of size of seed from constant temperature treatments were:

55-55-55	0.3405 gm.
70-70-70	0.2978 gm.
85-85-85	0.1724 gm.

The rapeseed from the intermediate (70° F) and higher (85° F) temperature treatments were 12.5% and 49.4% smaller, respectively, than the seeds from the low (55° F) temperature treatment. Thus the depressing effect of high temperature on seed size was much greater than that of an intermediate temperature.

TABLE III   Size of rapeseed (grams per 100 seeds) from temperature – time treatments of Exp. I

Treatment[1]/ Degrees F.	X-X-55	X-55-55	X-X-70	X-70-70	X-X-85	X-85-85	Row mean
55-55-X	.3405		.2986		.2179		.2857
55-X-X		.3405		.2875		.1409	.2563
70-70-X	.3738		.2978		.3138		.3285
70-X-X		.3685		.2978		.1507	.2723
85-85-X	.4195		.3406		.1724		.3108
85-X-X		.4142		.3201		.1724	.3022
Column Mean	.3779	.3744	.3123	.3018	.2347	.1547	

1/   Treatments are coded as indicated in a three term expression X-X-X representing temperature treatments during initial and terminal periods. The middle term can be part of either initial or terminal period, depending on duration of initial period.

Variable Temperature Treatments

The variable temperature treatments in Exp. I were arranged so that each treatment consisted of only two different temperature periods (Table III). Thus for purposes of discussion the middle term of the three part expression can be combined with either the first or third period to give an 11-day initial or a 22-day initial period.

Temperature during terminal periods tended to be negatively associated with seed size. The ranges of seed size, in grams per 100 seeds, for the three terminal temperatures (long and short initial periods combined) reveal the nature

of the association:

X-X-85°F.	0.1409 - 0.3138
X-X-70°F.	0.2875 - 0.3406
X-X-55°F.	0.3405 - 0.4195

Except when followed by 85° F. terminal temperature there
was little difference between the effect of long ·and short
initial periods on seed size.   Seeds from 22-day initial
(55° F. or 70° F.) and 85° F. terminal treatments were approx-
imately twice the size of those from the corresponding 11-day
initial periods (Table III).

Temperatures during long and short initial periods of
the three temperatures were not closely associated with seed
size.   The wide ranges of seed size for the three initial
temperatures which follow illustrate this point:

85°F.-X-X	0.1724 - 0.4195
70°F.-X-X	0.1507 - 0.3738
55°F.-X-X	0.1409 - 0.3405

The overlapping of these ranges suggest that initial temper-
atures have much less effect on seed size than terminal
temperatures.

The effect of combinations of initial and terminal
temperatures must also be considered.   Study of Table III
reveals that a high temperature followed by an intermediate
or low temperature and an intermediate temperature followed
by a low temperature invariably resulted in larger seed than
the corresponding intermediate or low temperature for the

entire period from bolting to maturity. The data in Table III
also shows that a low temperature followed by a high tempera-
ture resulted in smaller seed than the corresponding low or
intermediate temperature used for the entire period from
bolting to maturity. Thus the results of the variable temp-
erature treatments can be summarized as follows: A period
of relatively high temperature followed by a period of lower
temperature tends to increase seed size while a period of
relatively low temperature followed by a period of higher
temperature tends to decrease seed size.

Experiment II

Constant Temperature Treatments

The results of the constant temperature treatments
(Table IV) in this experiment confirmed results obtained in
Exp. I. The effect of temperature on size of rapeseed is
illustrated by:

60-60-60	0.3376 gm.
70-70-70	0.3060 gm.
80-80-80	0.2292 gm.

The seeds from the intermediate temperature (70° F.) and
high temperature (80° F.) were 6.5% and 32.2% smaller,
respectively, than seeds from the low (60° F.) temperature
treatment. The range of temperatures was smaller than in
Exp. I so the smaller ranges in seed sizes was expected.
The strong negative association between seed size and a