MONTANA STATE UNIVERSITY

Sex ratio studies on the wheat stem sawfly, Cephus Cinctus Nort
by Arthur James McGinnis

A ThESIS Submitted to the Graduate Committee in patrial fullfillment of the requirements for the degree of Master of Science in Entomology
Montana State University
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Abstract:
The investigations described were conducted at the Field Crop Insect Laboratozy, Lethbridge., Alberta and Montana State College, Bozeman, Montana, Data are presented from controlled experiments which confirm field observations that the sex ratio of progeny of mated Cephas clnetus females is influenced by the host plant variety, Sawfly populations reared from Thatcher had a greater proportion of males than did those reared from Red Bobs. The data also indicate that virgin females produced male progeny almost exclusively, irrespective of the host variety.

A study of sex ratio of progeny from individual females was only partly successful because of limited oviposition. Normal oviposition was not obtained under the controlled conditions used, The hypothesis that selective fertilization during oviposition influences sex ratio received some support but the data were too limited for conclusive evidence. A theory of differential mortality is suggested to account for the varietal influence on sex ratio.

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SEX RATIO STUDIES ON THE WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORT.

by

ARTHUR JAMES McGINNIS

A THESIS

Submitted to the Graduate Committee

in

partial fulfillment of the requirements

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Master of Science in Entomology

at

Montana State College

Approved:

Signed: John A. Callenbach
In Charge of Major Work

Signed: John A. Callenbach
Chairman, Examining Committee

Signed: J. A. Nelson
Chairman, Graduate Committee

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ABSTRACT

The investigations described were conducted at the Field Crop Insect Laboratory, Lethbridge, Alberta and Montana State College, Bozeman, Montana.

Data are presented from controlled experiments which confirm field observations that the sex ratio of progeny of mated Cephus cinctus females is influenced by the host plant variety. Sawfly populations reared from Thatcher had a greater proportion of males than did those reared from Red Bobs. The data also indicate that virgin females produced male progeny almost exclusively, irrespective of the host variety.

A study of sex ratio of progeny from individual females was only partly successful because of limited oviposition. Normal oviposition was not obtained under the controlled conditions used.

The hypothesis that selective fertilization during oviposition influences sex ratio received some support but the data were too limited for conclusive evidence.

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SEX RATIO STUDIES ON THE WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORT.

INTRODUCTION

The wheat stem sawfly, *Cephus cinctus* Nort., has been a serious pest in the wheat fields of the great plains region for a number of years. *C. cinctus* was first reported mining grass stems near Alameda, California, in 1890 (1). In 1895 its presence was reported from two points in the Canadian prairies (10). The last fifty years have seen this insect reach widespread importance as a threat to the prairie wheat-growing industry.

As the acreage under cultivation increased in the plains region the sawfly became firmly established as a parasite of the wheat plant. During the late 'thirties' and early 'forties', millions of bushels of wheat per year were lost owing to the ravages of this insect. A number of factors are responsible for the sawfly population reaching economic proportions but three are of major importance.

The development of rust-resistant wheat varieties contributed largely to the build-up of the population. Prior to this development the sawfly population had been virtually eliminated following each rust epidemic. When resistant wheat varieties replaced the susceptible ones in prairie agriculture the rust epidemics ceased to occur. Thus the sawfly population escaped the devastating effects occasioned by these epidemics and continued to thrive.

To reduce losses from wind erosion new cultural methods were applied. While the new practices provided control for wind erosion they afforded little control over the sawfly. The mouldboard plough had effectively controlled the sawfly population through burying the stubs. With the introduction of surface tillage implements, this control was lost and the sawfly situation became more critical.

As a further measure in the control of soil drifting, strip farming was introduced. This change in practice immediately increased the losses caused by the wheat stem sawfly. Usually only the margins of wheat fields suffer serious loss. With large blocks being replaced by narrow strips, the losses increased in the same ratio as did the field margins; where very narrow strips were used, infestations upward of 90 per cent occurred.

As the severity of the infestations increased adequate control measures were sought. Cultural controls proved only partially satisfactory. Accordingly, in 1932, a co-operative project between the Cereal Division at the Dominion Experimental Station, Swift Current, Saskatchewan, and the Dominion Entomological Laboratory, Lethbridge, Alberta, was initiated to study sawfly resistance in wheat and to develop a resistant variety.

Solid-stemmed varieties of Triticum vulgare and Triticum durum showed marked resistance to sawfly attack (14). Consequently a breeding program was initiated in which it was hoped to combine stem solidness with high quality thus producing a suitable resistant variety.

Hybrid lines were selected and tested for resistance to wheat stem sawfly attack. Replicated uniform nurseries were established at various points in the sawfly-infested regions of the Canadian prairies. The index of resistance was based on two factors, extent of cutting and percentage emergence. Emergence data collected on all lines and varieties grown each year showed that some varieties modified the sex ratio of the emerging populations.

Although the sex ratio of C. cinctus populations from most bread wheat varieties showed an equal number of males and females, the variety, Red Bobs, consistantly produced a preponderance of females. Irrespective of nursery location or crop year Red Bobs produced approximately two females to every male. This consistent deviation in sex ratio occasioned by Red Bobs suggested the possibility that host variety influences the sex ratio of the parasite. Thus it was suggested that a study of the factors influencing the sex ratio of C. cinctus populations would be very worthwhile.

This study was undertaken in an effort to determine what factors influenced sex ratio and to explain how the host variety influences the sex ratio of the parasite.

REVIEW OF LITERATURE

The diverse sex ratios of C. cinctus populations from bread wheat varieties indicate that some factor or factors peculiar to those varieties have influenced the resultant sex ratio. Thus the determination of sex in C. cinctus appears to be in part a function of the environment.

The problem of sex determination has been studied by workers for many years. McClung (16) suggested that the unpaired or accessory chromosome found in cytological examination of certain Orthopterans is important in sex determiniation and writes, "It is very probable that, in certain species, sex is determined at the time of fertilization and cannot be altered by any later influences. Conversely, it has been experimentally proved that the proportion of sexes may be materially altered by changed nutritive conditions operating upon larval forms, or may possibly be changed several times in the same individuals."

Holdaway (12) and Holdaway and Smith (13) report one of the most striking instances of nutrition influencing the sex

ratio of a populations. Working with the confused flour beetle, Tribolium confusum Duval, it was found that starvation immediately after hatching for varying periods of time materially changed the sex ratio of the resulting adult populations.

Herms (11) found that food supply during the early larval stage influences the sex ratio of adult populations. Underfed populations of the greenbottle fly, Lucilia sericata Meigen, showed a preponderance of males while well fed populations were predominatly females. With mosquitoes, Theobaldia incidens Thom., the underfed populations were largely females and the well fed were males. Thus it appears that starvation can be an important factor in sex determination but prediction of the predominant sex is difficult.

Environmental factors, other than nutrition, also have been shown to affect the sex ratio of populations. Clausen (4) from his studies of hymenopterous parasites lists several environmental factors which influence the proportion of males to females in a population. Among these are listed, (1) different years with the host population increasing or decreasing rapidly, (2) different geographical regions and (3) different hosts.

Brunson (2) working with the hymenopteran, Tiphia popilliavora Rohwer, reports a difference in the sex ratio

of populations emerging from Japanese beetle larvae parasitized in the second as compared with third-instar. Males predominated in the population emerging from second-instar host larvae while females predominated in those populations emerging from third-instar larvae. Further studies (3) provided proof that this variation was the direct result of the ability of the parent female to control the sex of her progeny at the time of oviposition.

Flanders (6) suggests that certain stimuli which activate the spermatheca of the parent females influence the sex ratio of the progeny. The spermatheca, subject to the required environmental stimulus, thus acts as a sex-determining mechanism at the time of oviposition. If the oviposition rate is high or if oviposition occurs on an unpreferred host not supplying the required stimulus, spermathecal activity is inhibited, resulting in a high proportion of males.

Flanders (9) lists as three factors which may alter the sex ratio of a population of Macrocentrus ancylivorous, excessive heat, light and parasite density. These factors may affect the impregnation and preoviposition period of the female through their influence on placement of the spermatophore. Should the female be inadequately inseminated or if both impregnated and virgin females are competing for the same hosts, male progeny will likely predominate. Similarly, Flanders (7) suggests that time of mating, in

species biparental for one sex, is likely to influence the sex ratio of the progeny.

Speicher and Speicher (20) found in cytological studies of Habrobracon that unfertilized eggs which produce females are tetraploid before reduction division. Further to this Flanders (8) has found that the level of nutrition during the larval period influences the type of ovarian tissue in the adult female. The amount of tetraploid tissue present is directly related to larval nutrition. Hence well fed females were thelytokus and underfed females were arrhenotokus. In this fashion larval nutrition is important in fixing, within limits, the sex ratio of the coming generation.

Recently Mackay (15) examined cytologically a limited population of C. cinctus. Her results indicate a diploid female and haploid male

Generally it may be stated that sex determination is a complex phenomenon and in the wheat stem sawfly it is possible that an interplay of factors controls the sex ratio of a population from any given variety. Parthenogenetic reproduction is known to occur in C. cinctus (5,21). Biparental reproduction is also recognized. These two conditions undoubtedly contribute to the final sex ratio of a given population. Furthermore, the wheat stem sawfly is obliged to complete its development within a single stem,

having no opportunity to move from an unfavorable environment. Consequently it may be expected that the host plant very markedly influences the developing parasite. The question then arises whether the influence of the host plant is sufficient to alter the sex of the parasite.

PART I

BULK POPULATION STUDIES

A number of factors were suspected of contributing to the modification of sex ratios. Consequently an exploratory experiment was designed to test some of the suspicions.

It was first necessary to confirm field observations under controlled conditions. By confining female populations over the two varieties selected it was possible to determine whether the sex ratio differences were inherent in the varieties or whether these differences resulted from host selection by the parent populations.

Evidence in the literature (11, 12, 13) indicated that the level of nutrition during the early stages affected the sex ratio of the resultant population. In testing this possibility in C. cinctus it seemed reasonable to assume that in different varieties the available nutrients for the developing larvae were not entirely the same. Further it was possible that the nutritive

qualities were different enough to influence sex determination in the parasite.

Because of the intimate association between the host and the parasite and the insect's inability to escape from an unfavorable environment, it was considered possible that within a variety different stems offered a wide enough variation in the nutrition of the developing larvae to influence sex. Type of stem, age of stem and period of caging the stems were three factors studied in an effort to determine whether individual stem differences were important.

Because the main stems are more vigorous it was thought that populations developing in main stems might be subjected to a more sutiable environment than those developing in tillers. Consequently it was proposed to keep the two populations separate so this possibility could be explored.

The maturity of the stems in which development of the parasites occurred was also suspected of being influential in determining the sex of the developing parasite. Two methods of studying this factor were proposed:-

(1) Different dates of seeding with one infestation date would give a range in age of the stems tested.

(2) Different dates of infestation with one seeding date would give a similar range in age of the stems tested.

The only suitable method of confining sawflies to small plots is with cages. Further, if a wild population is present,

the cages act as a barrier to contamination of the experimental material. As wheat plants grown within cages are noticeably different from those grown without cages, the possibility of nutrition influencing sex again appears. In an exploratory study of this type it seemed advisable to consider this factor.

Aside from the nutritional aspects, the study of one other factor seemed necessary. What role does mating have in sex determination and the ultimate sex ratio of C. cinctus population? Evidence is presented in the literature (3) showing that in some instances sex can be controlled by the female at the time of oviposition. A study of the role of mating seemed necessary, for it would indicate, in part, the relative importance of nutrition and selective fertilization in determining the sex ration of a sawfly population.

MATERIALS AND METHODS

Red Bobs and Thatcher, two varieties of wheat known from field observation to yield populations with different sex ratios were chosen as host material for this work. Registered seed of each variety was used.

A Planet Junior garden seeder, adjusted to drop approximately 24 kernels per row foot, was used to seed the nursery.

Screen cages, two feet square and 30 inches high were used for confining the sawfly populations to the test plots.

The parent sawfly material was collected from a test nursery near Regina, Saskatchewan, during the first week in May, 1948, and was retained in the laboratory at Lethbridge under controlled conditions. On arrival at Lethbridge approximately 3000 were isolated in individual 21 x 70 mm. vials, stoppered with absorbent cotton.

A randomly chosen sample of these vialed stubes was placed in a 25° C. cabinet until emergence was noted. The balance were placed in a 10° C. cabinet to retard development. In this manner, an index of time required to emergence was obtained and it was possible to regulate mass emergence so that adequate populations were available at the required stage of development of the wheat in the nursery.

Figure 1 depicts the experimental design and indicates the environmental factors being tested. The two varieties were sown in alternate rows with six-inch spacing. Each cage thus enclosed eight row feet, composed of two 2-foot rows of each variety. The west row in each cage was Thatcher. The cages were set two feet apart in each direction and a three-foot margin was left on all sides of the area being caged.

Wheat was sown on three different dates, May 5, 13, and 21. Infestation followed on June 25, July 3 and 9.

The sawflies were emerged and the required number mated the day prior to infestation. Mating was accomplished by placing

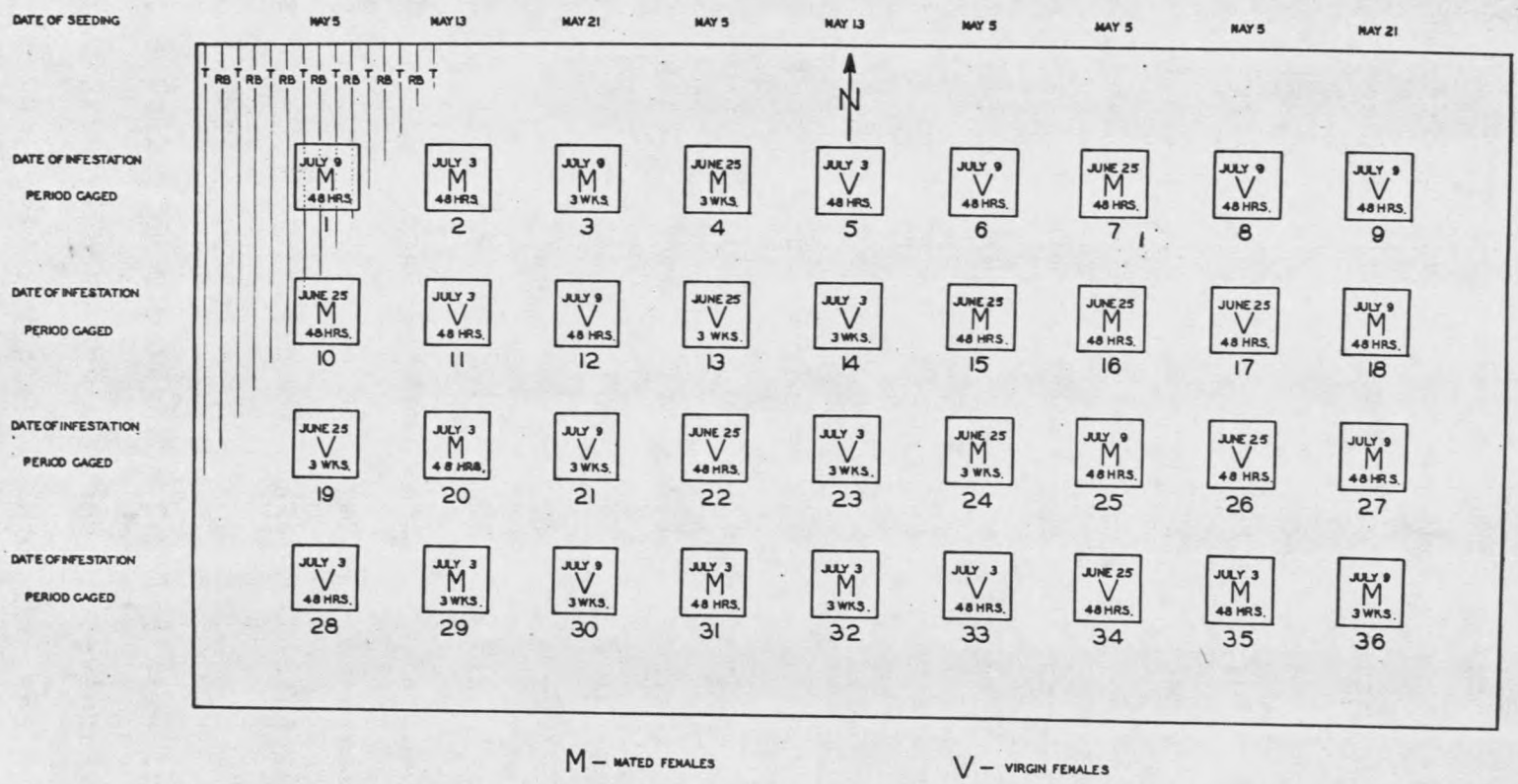


Figure 1 - Plot Design

by placing a randomly-selected male in a vial containing an adult female. The vials in which mating occurred were stoppered with corks. Absorbent cotton proved unsatisfactory for, as activity increased, the insects became entangled in the cotton fibres.

The vials containing the pairs of insects were laid on large metal trays and placed out-of-doors where the stimulus for mating seemed to be greatest. Short periods in direct sunlight were apparently not deterimental to the insects but intense sunlight over prolonged periods markedly reduced activity and eventually caused death. Mating occurred most readily in a partially shaded region where there was an interplay of direct sunlight and shadow.

When copulation was observed, the vial containing the pair was gently removed from the tray and placed in the shade. As soon as possible after the copulating pair separated, the females were removed from the vials and placed in lots of twenty-five in one-pint glass "Vacu-Top" jars. Each jar contained a small mass of moistened excelsior and was covered with a double layer of white cheesecloth. Lots of twenty-five virgin females were also selected and placed in similar containers.

The following morning the females were transported to the nursery site, where the cages had previously been arranged over the necessary plots. Each jar was placed in the center of a

cage and the cheesecloth covering removed. The insects of the first-infestation series were permitted to escape at will. After twenty-four hours it was found that some individuals still remained in the jars and on subsequent infestations the females were shaken out of the containers.

Twelve of the 36 test plots were infested on each of three dates, June 25, July 3 and 9. Of these twelve, six were infested by a virgin population and six by a mated population. To determine the effect of caging, four of the twelve plots per infestation date were caged from June 24 to July 12. The remaining eight were caged for 48 hours only. In all cases the females were left in the cages for forty-eight hours and then removed with an aspirator, the air pump being powered by a small portable gas motor.

Following cutting in the fall the stubs were collected and stored in one-pint "Vacu-Top" containers which were half filled with moist sandy soil.

EXPERIMENTAL

Oviposition

Prior to the first and second infestations egg counts were made on samples of 25 females as an egg-complement index. Thus it was possible to determine the effectiveness of the 48-hour oviposition period by counting the eggs remaining in the females.

The average number of eggs per females for Series 1 was 43 and for Series 2, 29. It is likely that the egg count on the second series of females is low because it was made after a number were dead. Based on these figures, it is probable that the average egg complement of the females was upward of 35.

Table I shows the number of females recovered following each forty-eight-hour infestation period and the average egg complement. It shows that approximately 75 percent of the females released in the cages were recovered. This incomplete recovery offered a source of error to the experiment as a whole. Of the unrecovered individuals, it is likely that some were dead but undoubtedly some had excaped. The likelihood of these escapes seriously influencing the results is small, as wheat stem sawflies tend to congregate along the margins and a three-foot border was provided around the test region of the nursery.

For purposes of comparison it is assumed that the average egg complement of the female population was 30, a figure which is certainly low. Since this is a minimum, calculations made as to eggs laid per plot will be, in all probability, lower than the absolute values but the figures should be comparable. Table II shows the relationship between the number of eggs laid per plot and the percentage cutting data.

TABLE I

Number of females recovered per plot following 48-hour-infestation period and average egg complement at time of recovery

Series 1			Series 2			Series 3		
Plot No.	No. of Females Recovered	Mean No. of Eggs/Female	Plot No.	No. of Females Recovered	Mean No. of Eggs/Female	Plot No.	No. of Females Recovered	Mean No. of Eggs/Female
Mated Females								
4	20	-	2	21	7.3	1	20	16.0
7	12	-	20	22	19.2	3	18	20.2
10	21	-	29	20	3.3	18	19	23.2
15	18	-	31	22	6.1	25	16	21.3
16	19	-	32	20	6.2	27	16	29.0
24	20	-	35	21	13.9	36	22	22.7
Mean	18	-	Mean	21	9.2	Mean	18	21.9
Mean%	74		Mean %	84		Mean %	74	
Virgin Females								
13	21	-	5	20	4.0	6	14	16.1
17	12	-	11	21	15.2	8	23	19.9
19	20	-	14	15	5.5	9	19	22.6
22	25	-	23	20	9.2	12	18	17.0
26	16	-	28	21	0.0	21	17	31.0
34	22	-	33	20	9.8	30	19	23.1
Mean	19	-	Mean	20	7.0	Mean	18	21.7
Mean %	77		Mean %	78		Mean %	73	

TABLE II

Mean number of stems, mean percentage cutting, and estimated number of eggs laid, per plot

Series	Females	No. of Stems/Cage	Estimated No. Eggs	Eggs/Stem	Percentage Stems Cut
2 . .	Mated	191	520	2.7	41.4
	Virgin	202	575	2.8	31.0
3 . .	Mated	192	202	1.0	50.2
	Virgin	194	208	1.1	46.1

From Table II it appears that sufficient eggs were laid by the female population to infest every stem. As it is doubtful that all stems offered suitable oviposition sites, it is assumed that a number contained more than one egg at the end of the 48-hour period.

Table II also indicates that virgin females laid eggs as readily as did the mated females but fewer stems were cut. No explanation is offered for this phenomenon.

Cutting

Periodic observations were made for nearly a month following the last infestation and subsequent removal of cages from the test plots. Signs of active cutting were first noted on August 11. At this time the plots were trimmed and all the stems in the intervening spaces were removed to prevent tangling when cutting became general.

Percentage cutting, by plots, was estimated on August 19 and 26. Cutting was completed by September 9 and the stubs were lifted. Percentage cutting was determined in each of the four rows per test plot by counting the number of standing and cut stems. The stubs were further separated as to main stem or tiller orgin and percentage cutting determined in each case. Thus eight groups of stubs were obtained from each plot. The identity of each group was maintained until emergence occurred.

The relationship between infestation date and time of cutting is given for each plot in Table III. Regardless of infestation date approximately the same period of time, 60-65 days, was required for larvae to reach the cutting stage.

Table III shows that while approximately the same length of time was required for the larvae to reach maturity, later oviposition resulted in slightly more rapid development, coincident with more rapid rate of plant maturity.

The data on cutting were too cumbersome to be presented in their entirety. Table III gives the data by complete plots but gives no indication of differences within plots. Consequently the mass of data gathered on cutting was subjected to an analysis of variance, presented in Table IV. The analysis confirmed nursery observations that Thatcher was more severely cut than Red Bobs. This difference proved

TABLE III

Percentage cutting by *C. cinctus* mated and virgin females in individual plots on August 19, August 26 and September 9, 1949*

Date of Infestation	Plot No.	Percentage cutting in plots infested by mated females			Plot No.	Percentage cutting in plots infested by virgin females		
		Aug. 19	Aug. 26	Sept. 9		Aug. 19	Aug. 26	Sept. 9
June 25	4	50	50	46.6	13	40	35	44.2
	24	45	45	50.8	19	40	40	42.8
	7	45	50	58.9	17	20	50	52.4
	10	35	35	48.3	22	35	30	37.2
	15	15	30	33.1	26	35	35	46.9
	16	45	60	63.1	34	30	45	59.0
	Mean	40	45	50.1	Mean	33	40	47.1
July 3	31	5	40	53.9	28	10	25	30.3
	35	4	40	61.5	33	8	20	28.4
	29	4	20	32.7	14	1	10	24.6
	32	8	35	35.1	23	5	15	33.1
	2	3	25	40.8	5	2	15	25.4
	20	0	5	12.7	11	4	20	47.5
	Mean	4	28	39.4	Mean	5	18	31.6
July 9	1	5	35	53.5	6	3	10	31.0
	25	3	35	40.7	8	1	40	56.0
	3	0	45	56.4	21	0	25	54.8
	36	0	20	46.8	30	1	25	44.8
	18	0	10	56.0	9	0	15	49.4
	27	0	10	51.4	12	0	25	43.5
	Mean	1	26	50.8	Mean	1	23	46.6

* Percentage cut on August 19 and August 26 are estimates only, but cutting on September 9 is by actual count at time stubs were lifted.

to be statistically significant. By count, 48.0 percent of the Thatcher stems were cut, compared with 41.4 percent of the Red Bobs.

After cutting had begun it was obvious that some replicates were more severely cut than others. The experiment was replicated four times, with each plot constituting two replicates of a particular treatment. Thus two plots were required to replicate a treatment four times. The two west rows in each plot were more severely cut than the two east rows of the same plot. Hence replicates 1 and 3 were more severely cut than 2 and 4. An actual count revealed the cutting by replicates to be: Replicate 1 - 50.2%; Replicate 2 - 39.1%; Replicate 3 - 50.3%; Replicate 4 - 39.1%. This marked variation between replicates indicates a positional influence as to the plants infested within a plot. To date there is no explanation for this.

Even more striking is the cutting gradient found to exist within the plots. Considering the west row of each plot as No. 1 and moving east in rotation the average cutting by rows was found to be Row 1 - 53.1%; Row 2 - 45.5%; Row 3 - 41.4%; Row 4 - 35.4%. Since in all cases Thatcher was the west row in each plot, the position effect of the row within the plot as shown above complicated interpretation of the cutting data. The question arises as to the relative importance of variety and row position with respect to cutting. Since

TABLE IV

Analysis of variance of percentage cutting by C. cinctus in wheat test plots

Variance due to	D. F.	S. Sq.	M. Sq.	F.
Variety	1	3176.05	3176.05	91.93**
Replicates	3	8935.81	2978.60	86.21**
Error I	3	103.64	34.55	
Intermediate Total I	7	12215.50		
Mating	1	1410.69	1410.69	2.99
Mating x Variety	1	0.25	0.25	
Error II	6	2834.12	472.35	
Intermediate Total II	15	16460.56		
Stems	1	8458.67	8458.67	7.93*
Stems x Variety	1	374.46	374.46	3.51
Stems x Mating	1	197.67	197.67	1.85
Stems x Variety x Mating	1	341.93	341.93	3.21
Error III	12	1280.09	106.67	
Intermediate Total III	31	27113.38		
Treatment	8	15468.56	1933.57	2.35
Treatment x Variety	8	4273.96	534.24	2.40**
Treatment x Mating	8	6578.03	822.25	3.70**
Treatment x Stems	8	2031.69	253.96	1.14
Treatment x Variety x Mating	8	878.24	109.78	0.49
Treatment x Variety x Stems	8	1574.36	196.80	0.88
Treatment x Mating x Stems	8	1170.97	146.37	0.66
Error IV	200	44494.47	222.47	
Total	287	103583.66		

**Significance at the 1% point. *Significance at the 5% point.

the rows were not randomized within each plot, it is recognized that the analysis of the cutting data is subject to certain criticism.

Under field conditions Red Bobs is less severely cut than are most other hollow stemmed bread wheat varieties, including Thatcher (18). Thus it is assumed that the varietal difference as shown in the work is real and is not entirely the result of this unexpected position effect.

Table IV indicates a significant difference in the percentage cutting of main stems as compared with tillers. The data showed 50.1% of the main stems were cut while only 39.3% of the tillers were severed. This is the usual field observation and is attributed to the fact that at the time of oviposition the main stems offered more suitable oviposition sites.

No statistically significant differences appeared between treatments but two treatment interactions are important. Included in treatments are, dates of seeding, dates of infestation and periods of caging.

The treatments used and the average percentage cutting by variety are given in Table V which shows that cutting of both varieties was significantly less in those plots sown May 13 than in plots sown either earlier or later. This occurred although the plants were of equal age at time of infestation.

TABLE V

Average percentage cutting of each variety according to treatment

Treatment No.	No. of Days Caged	Date of Seeding	Date of Infestation	Age of Plant Upon Infestation (in days)	Percentage Cut	
					Thatcher	Red Bobs
1	18	May 5	June 25	52	48.9	45.2
2	2	" 5	" 25	52	51.3	53.1
3	2	" 5	" 25	52	53.0	53.8
4	2	" 5	July 3	60	51.0	35.2**
5	2	" 5	" 9	66	53.7	34.4**
6	18	" 13	" 3	52	33.0**	34.0**
7	2	" 13	" 3	52	39.0**	23.4**
8	18	" 21	" 9	50	49.7	46.7
9	2	" 21	" 9	50	52.5	46.5

Minimum significant difference = 7.5%

** Significance at the 1% point.

It is also interesting to note in Table V that Red Bobs plants beyond a certain stage of maturity seem either to offer a less suitable environment to the developing parasite or inhibit oviposition, for less cutting occurred in these plots.

Table VI shows the average percentage cutting of plots infested by virgin and mated females according to treatment. These data also show the lowerpercentage of cutting in plots sown May 13. They further indicate that Red Bobs plots 60 and 66 days of age when infested by virgin females suffered less cutting.

Emergence and Sex Ratio

Salt (19) describes the conditions found most suitable for breaking diapause in C. cinctus and these were duplicated as closely as possible. All the stubs were separated and the roots removed. Moist sandy soil was placed in the bottom of pint "Vacu-Top" jars and the stubs were dispersed in the containers in such a manner that individual stubs were entirely surrounded by the moist soil. The jars were then sealed with rubber rings to prevent moisture loss. On September 20 the containers were placed in a refrigerated room held at 40° F. They were transported to Bozeman, Montana, December 21 and the next day were placed in a warm room, 77-80° F., for post diapause development.

First emergence was noted January 19, four weeks after the stubs were raised to the higher temperature. The

TABLE VI

Average percentage cutting in plots infested by mated and virgin C. cinctus females according to treatment

Treatment No.	No. of Days Caged	Date of Seeding	Date of Infestation	Age of Plant Upon Infestation (in days)	Percentage Cut	
					Mated	Virgin
1	18	May 5	June 25	52	47.9	46.2
2	2	" 5	" 25	52	55.7	48.7
3	2	" 5	" 25	52	50.8	56.0
4	2	" 5	July 3	60	56.4	29.7**
5	2	" 5	" 9	66	46.1	41.9
6	18	" 13	" 3	52	36.5**	30.5**
7	2	" 13	" 3	52	26.9**	35.6**
8	18	" 21	" 9	50	47.9	48.4
9	2	" 21	" 9	50	53.9	45.2

Minimum significant difference = 7.5%

** Significance at the 1% point.

soil was sifted from the stubs and the rubber rings were removed at this time. Records of emergence were taken every second day until January 31. On February 7 each container was again checked for late emerging adults but very few were found. Following this last observation, the stubs were split and the adults, dead within the stub, were sexed and recorded. A record was also taken of those larvae which had failed to pupate and those which were dead.

Final results showed 85% emergence, 8% mortality and 7% larvae. Generally, Thatcher showed a slightly lower percentage of mortality and non-developing larvae than did Red Bobs but the differences were small and perhaps not real. The close relationship between temperature and moisture conditions to which the stubs were subjected and the breaking of diapause suggests that lack of uniformity in conditions was responsible for these discrepencies. The possibility of potential differences within individual larvae existed but time did not permit reconditioning the remaining stubs so the larvae were neglected in sex ratio analysis. The sex ratio data presented herein are thus based on 85 percent of the total population.

Previous work at Lethbridge had indicated 85 percent emergence was exceptional; the usual emergence was close to 60 percent. Further, data have been accumulated indicating that the sex ratio of a population based on 60 percent emergence

is a good indication of the sex ratio for the whole. Thus the differences occuring in the data on sex ratio are assumed to be real.

Table VII shows the analysis of variance of numbers of each sex emerging from the experimental plots infested by mated females. The number of adults emerging from the two varieties is shown to be significantly different. Because the analysis was based on numbers of adults, this difference is a direct reflection of the less extensive cutting in Red Bobs. Similarly the interaction, Treatment x Variety, although shown to be significant is not important here as it is a direct result of differences in cutting.

The sex ratio of the populations emerging from Thatcher and Red Bobs are significantly different as is evidenced by the highly significant interaction, Sex x Variety. As shown in Table VIII the population of 712 adults emerged from Thatcher yielded a ratio of 2.20 males per female; from Red Bobs, 513 adults emerged with a ratio of 1.05 males per female. Even when the 15 percent of unsexed individuals were added to the known populations in such a manner as to eliminate the difference the same general trend exists.

Table IX shows that the progeny of virgin females is almost entirely male. A few females are present and it is possible that some of the unrecovered mated females caused

TABLE VII

Analysis of variance of number and sex of progeny from wheat plots infested by mated C. cinctus females

Variance due to	D. F.	S. sq.	M. sq.	F
Variety	1	138	138.0	16.6*
Replicates	3	201	67.0	8.1
Error I	3	25	8.3	
Intermediate Total I	7	364		
Stems	1	10	10.0	3.2
Stems x Variety	1	4	4.0	1.3
Error II	6	19	3.1	
Intermediate Total II	15	397		
Sex	1	275	275.0	1.2
Sex x Variety	1	225	225.0	79.5**
Sex x Stems	1	6	6.0	2.1
Sex x Variety x Stems	1	11	11.0	0.4
Error III	12	34	2.8	
Intermediate Total III	31	948		
Treatment	8	393	49.1	1.9
Treatment x Variety	8	209	26.1	3.5**
Treatment x Stems	8	72	9.0	1.2
Treatment x Sex	8	139	17.4	2.3
Treatment x Variety x Stems	8	28	3.5	0.5
Treatment x Variety x Sex	8	47	5.9	0.8
Treatment x Sex x Stems	8	49	6.1	0.8
Treatment x Variety x Stems x Sex	8	21	2.6	0.4
Error IV	192	1440	7.5	
Total	287	3346		

* Significance at 5% point. ** Significance at 1% point.

TABLE VIII

Number of C. cinctus and sex ratio of populations emerging from Thatcher and Red Bobs stems infested by mated females

	Variety			
	Thatcher		Red Bobs	
	No. of Stubs	No. of Individuals Emerged	No. of Stubs	No. of Individuals Emerged
Total	802	712	622	513
Males		490		263
Females		222		250
Males / Females		2.20		1.05

this discrepancy by laying fertilized eggs within these test plots. However, the females may be the result of tetraploidy in the ovarian tissue of the virgin females or the usual reduction division not occurring. Furthermore a larger number of females emerged from the Red Bobs stubs, Table IX. This substantiates the premise that in some mamner Red Bobs tends to produce a preponderance of females.

TABLE IX

Number of males and females in progeny of virgin C. cinctus females according to variety of wheat and type of stem

	Variety			
	Thatcher		Red Bobs	
	No. of Individuals from		No. of Individuals from	
Sex	Main Stems	Tillers	Main Stems	Tillers
Male	294	271	202	243
Female	2	3	10	7

Discussion

Of the six factors tested, only mating and variety appeared to affect sex ratio of C. cinctus populations. Over 97 percent of the progeny of virgin females were males. Although the progeny of mated females showed a preponderance of males, it was much less extreme. Thus, mating seems to be necessary if both sexes are to be produced in approximately equal numbers.

Field observation had indicated relatively constant sex ratios for populations emerging from the two varieties, Thatcher and Red Bobs. These were 1:1 and 1:2 males per female, respectively. Under the conditions described the sex ratios of populations emerging from Thatcher and Red Bobs infested by mated females were 2:1 and 1:1 respectively. Maleness is more pronounced in populations from both varieties but the same relative trends in sex ratio are present and a greater proportion of females in the population reared from Red Bobs was present.

Why the increased proportion of males in the progeny of mated females should occur is not known. Two postulates are presented to account for this condition. First, each of the mated females was permitted to mate only once prior to their release in the test plots. Perhaps more than one mating during the oviposition period is required for

adequate insemination. Second, while all the females were observed to copulate it is not known if they were all adequately inseminated. Had a number of the females believed to be mated received no sperm their progeny would be largely males.

As mentioned previously more males appeared in the progeny of mated females than was expected, but the sex ratios of sawflies developing in the two varieties showed the same relationship as observed in the field. Thus the varietal influence noted in field work was reproduced under controlled conditions. The mechanism through which variety influences the sex ratio of the developing population cannot be definitely established from this study. However, there is an indication that differential mortality may be responsible, in part, for the varietal differences in sex ratio. Table II shows that the virgin females apparently laid eggs in both varieties as readily as did the mated females yet fewer stems were cut. This indicates that either some of the eggs laid by the virgin females did not hatch or some of the larvae did not reach maturity.

Mackay (15) has shown that the female is diploid and the male haploid in chromosome content. The assumption seems valid that females are derived from diploid eggs and males from haploid eggs. Except for anomolous chromosome

behavior it is expected that the haploid male producing egg is unfertilized while the diploid female producing egg is fertilized. The fertilized female is therefore able to produce either males or females but the virgin female would be expected to produce males only. Although some anomolous chromosome behavior is indicated, Table IX shows the usual pattern of males from unfertilized eggs.

The data suggest that males have less resistance to adverse environmental conditions than do females. Undoubtedly a certain proportion of the male producing eggs laid by either virgin or mated females were deposited in stems not offering an ideal environment. If males have less resistance to adverse conditions, because virgin females laid the larger number of male producing eggs it would be expected that correspondingly fewer of the progeny of virgin females would reach maturity. Table VI supports this concept for cutting was less extensive in the plots infested by virgin females.

Of the two varieties used in this work fewer Red Bobs stems were cut, as shown in Table V. Perhaps the environment in Red Bobs stems is less suitable than that offered by Thatcher stems. Consequently, mortality is higher among the individuals parasitizing Red Bobs and fewer stems are cut. Of particular interest is the fact that in Red Bobs stems 60

and 66 days of age less cutting occurred than was noted in corresponding Thatcher stems and also in younger Red Bobs stems. It seems that the older Red Bobs stems provided a less suitable environment for development than did any of the other stems and that a larger proportion of the population died prior to larval maturity. Thus it seems likely that differential mortality between sexes accounts for the higher proportion of females in populations reared from Red Bobs.

PART II

STUDY OF INDIVIDUALS

In the experiment described above each test plot was infested with 25 females which provided sufficient progeny to give an evaluation of the interaction Sex x Variety. However, the sex ratios calculated for the populations from to two varieties were based on the offspring of over 400 females.

There was noticeable variability in size, vigor and egg complement among these females. Undoubtedly there were other differences, one of which might have been the inherent capacity to produce offspring with different ratios of males to females. Should this be the case the sex ratios of the progeny of individual females could be expected to

vary from extreme maleness to extreme femaleness. A second difference might have been the tendency to seek oviposition sites with certain specific qualities. Likely any given combination of favorable qualities would be found more often in stems of a single variety. Should the two be interrelated it is probable that females with an inherent female-producing capacity would tend to oviposit in a particular variety. Likewise oviposition by males-producing females would occur in another variety. The intermediate condition, where the emerging population is evenly divided as to sex, could occur in many ways. Should these suppositions be correct, variability among females is actually responsible for sex ratio differences.

Where one sex is produced uniparentally and females lay eggs before and after mating it is expected that time of mating will influence the sex ratio of the progeny (7). If an inadequate supply of sperm is received by the female or if the sperm are not viable or properly stored there will likely be a higher proportion of the uniparentally produced sex. Further, should sperm supply or storage be inadequate, female progeny would be expected from only the first eggs laid.

An experiment was conducted during the summer of 1948 to study the progeny of individual females.

MATERIALS AND METHODS

The two wheat varieties, Red Bobs and Thatcher, were used as host material. Four rows of each were sown in the greenhouse on each of three dates, May 11, 16 and 21. Subsequent plantings were made in five-gallon, stone-ware crocks to provide host material for the late summer.

Both virgin and mated females were used; emergence and mating being obtained in the manner described previously. When females were not under observation they were retained in the 21 x 70 mm. shell vials.

A small aspirator was used for handling the insects. When oviposition was desired, the females selected was taken from the vial and placed on what appeared to be a suitable stem and kept under continual observation. When an egg was deposited she was transferred to a suitable stem of the other variety. In this manner the same female laid eggs alternately in the two varieties.

Following oviposition, a label was attached to the base of the stem bearing information pertinent to the egg. For example a stem labelled 42M-RB-9, indicated that female number forty-two was mated and that the ninth egg laid by her was deposited in a Red Bobs stem. The tags were easily destoryed and as time permitted they were replaced by labelled wooden stakes.

As the stems were cut the stubs were lifted, individually packed in moist soil in shell vials, corked and placed in a 5° C. cabinet.

EXPERIMENTAL

Oviposition and Cutting

In this work a long series of eggs from each female was desired. First attempts to secure oviposition by individual females were made on June 20. These trials continued until August 23. Rarely did a female oviposit immediately after being placed on a stem and the rate of oviposition varied widely. Female No. 18 was observed to lay 14 eggs in two hours; No. 23, 12 in the same length of time. No. 25 laid seven in three hours while others refused to lay irrespective of the time allowed.

When a female would not oviposit in one stem, she was transferred to another. Occasionally this induced oviposition but more often there was no response. This modification in technique made it impossible to secure an equal number of eggs in the two varieties.

Table X shows the oviposition and cutting data obtained from the nine females laying the greatest number of eggs. These data show that nearly all the stems in which oviposition was recorded were cut. Of those which were uncut

most were broken or otherwise damaged during the growing period. This indicates that in very few instances did a female puncture a stem without depositing an egg. Hence observation of the female is apparently an accurate method of determining when and where an egg is laid.

Table X also shows that, on the average, mated females laid more eggs than did virgin females. Why this occurred is not known.

TABLE X

Number of eggs laid by mated and virgin C. cinctus females and number of stems of each variety cut

	Female No.	No. of Eggs Laid	Number of Stems Cut		
			Thatcher	Red Bobs	Total
Mated	3	29	16	11	27
	4	15	9	6	15
	18	14	7	7	14
	23	22	11	9	20
	25	18	10	7	17
	35	10	6	4	10
Virgin	26	10	6	4	10
	33	11	5	6	11
	40	6	3	3	6

Emergence and Sex Ratio

The stubs were retained in a 5° C. temperature cabinet from the latter part of September until the early part of April 1949. They were then placed in a 20 - 25° C.

room for post-diapause development. The soil was sifted from the stubs and the emerging adults were kept in the vials.

Table XI shows the emergence data collected on the progeny of the six mated females. Two interesting features appear in Table XI. First, the progeny of different females show variation in completeness of emergence. Only two adults emerged from 27 stubs all of which were cut by the progeny of female No. 3. Conversely of the progeny of No. 18, 12 out of 14 emerged.

Second, a very high percentage of the progeny was male. As the greater proportion of females are believed to develop from fertilized eggs it appears that the expected number of fertilized eggs were not laid. Placement of the spermatophore in Macrocentrus ancylivorus is governed by a number of factors (9). Although morphological details of the C. cinctus reproductive systym have not been worked out it is possible that the experimental techniques used prevented adequate insemination of the female.

It is noteworthy that females developed from eggs laid late in the respective series by females 18 and 23. Because it is not known whether the female progeny developed from fertilized eggs or were produced uniparentally, the role of insemination and sperm storage remains obscure.

TABLE XI

Effect of variety and order of oviposition on sex of progeny of individual, mated C. cinctus females

Egg No.	Female No. 3		4		18		23		25		35	
	T	RB	T	RB	T	RB	T	RB	T	RB	T	RB
1		-	M			M		F	M		-	
2	-			-	M		M			M		F
3	-		M			M		M	-		-	
4		-		-	M		M			M	F	
5	M		-			M		-	-			-
6		-		-	-		M		No stub		F	
7	-		-			M		-	M		M	
8	-		-		M		M			-		-
9		-		-		M	No stub		M		M	
10	-		-		M		M			M		-
11		-		-		M		M	M			
12	M		-		-		-			M		
13		-	-			F		-	-			
14	No stub			-	M		M			M		
15	No stub		-					M	M			
16	-						M			-		
17		-						-	-			
18	-						F		-			
19	-							-				
20	-						M					
21		-					No stub					
22	-						F					
23	-											
24		-										
25	-											
26		-										
27	-											
28		-										
29	-											

M = Male; F = Female.
T = Thatcher; RB = Red Bobs

The emergence data on the progeny of three virgin females are shown in Table XII but are too limited to be conclusive. The presence of a female produced by a virgin parent is noted. This suggests that some of the females listed in Table XI may have been produced uniparentally but an understanding of the role of mating in C. cinctus awaits detailed morphological and cytological study.

TABLE XII

Affect of variety and order of oviposition on sex of progeny of individual virgin C. cinctus females

Egg No.	Female No. 26		33		40	
	T	RB	T	RB	T	RB
1	-			M		M
2		M	-		-	
3	M			-		M
4		-	-		M	
5	-			-		-
6		M	M		-	
7	-			F		
8	M		-			
9	M			-		
10	-		-			
11				-		

M = Male; F = Female
T = Thatcher; RB = Red Bobs

Discussion

It was theoretically possible to compare the progeny of a female in two ways. First, the sex ratio of all her

progeny could be compared with the sex ratios of progeny of other females. Second, the sex ratio of the half of the progeny, developing in one variety, could be compared with that of the second half developing in the other variety. The former would show whether all females produced progeny with the same sex ratio and the latter if any varietal influence was exerted on the offspring.

Practially, these comparisons were impossible. First, too few females laid sufficient eggs, had they all developed to adults, to permit sex ratio comparisons to be made. Second, numbers were further reduced owing to the low emergence, 47.5 percent from Thatcher and 36.4 percent from Red Bobs. Third, the progeny of mated females showed a preponderance of males, 81.3 percent from Thatcher and 85.7 percent from Red Bobs, indicating that some factor or factors had modified the expected sex ratios.

The problem of obtaining oviposition seemed to be one of technique for under field conditions females often lay all their eggs prior to death. In the present study where the females were handled and transferred from stem to stem, an average of less than six eggs was laid per female. If studies of variation among the progeny of individual females are to be conducted successfully, techniques of handling

must be improved to secure almost complete oviposition under controlled conditions.

Extreme maleness has not been observed in field populations reared from these two varieties. Thus it appears that the experimental techniques of handling the parent population have contributed to the preponderance of males. The manner in which these techniques modified the sex ratios is not known.

PART III

MANUAL TRANSFER OF EGGS

The work described in Part I confirmed general field observations that the sex ratios of populations are modified by the variety in which development occurred. However, this study did not show the manner in which this influence operated. Likewise the study described in Part II shows the varietal influence on sex ratio but the mode of action is not clarified. A further study was undertaken to determine how this varietal influence occurred.

It has been shown that the female of the hymenopterous parasite, Tiphia popilliavora Rowher is capable of fertilizing eggs selectively at the time of oviposition; the stimulus for fertilization is apparently a function of

the size of the host larvae (2, 3). It is possible that C. cinctus females also possess the ability to selectively fertilize the eggs as they are laid; the stimulus for fertilization, by analogy, must arise from the host stem.

It has been suggested that fertilization of the egg during the act of oviposition may be influenced by the stance or grip the female maintains on the stem. Should this be the case, size and texture of the stem would likely be important in determining whether a haploid or diploid egg was deposited, with the stimulus for fertilization being provided by the stems in which oviposition occurred. A variety in which most of the stems stimulated fertilization would thus be expected to show a preponderance of females in the offspring; one having fewer stems which stimulated fertilization would show fewer females.

There is a marked difference in size of the stems of the two varieties, Thatcher and Red Bobs. It is likely that detectable differences in texture also exist. Thus it is possible that these differences between varieties account for the observed differences in sex ratio.

An experiment was conducted in 1949 to determine whether mated C. cinctus females laid more fertilized eggs in one variety than the other.

MATERIALS AND METHODS

The nursery was established on a plot adjacent to the Science Service Laboratory at Lethbridge, Alberta. It was divided into two separate units, the infestation-plot in which the female sawflies were permitted to oviposit and the transfer-plot into stems of which the eggs were manually transferred.

The infestation-plot consisted of 16 rows, 20 feet long, sown alternately to Thatcher and Red Bobs with 12 inch spacing. Four rows were sown on each of four dates, May 5, 12, 19 and 26. These four seeding dates permitted a longer period over which to conduct the study.

The transfer-plot was composed of 32 rows, 20 feet long, sown alternately to Thatcher and Red Bobs. The spacing used was alternately 12 and 24 inches. The 24 inch spaces between the rows were used as work areas when eggs were being transferred. In this plot eight rows were sown on each of the above dates.

The parent sawfly population came from the Regina, Saskatchewan area. The adults were emerged and the females mated using the techniques described previously. Infestations were made on four dates, July 4, 6, 11 and 13. Individual females were introduced into circular screen cages 12 inches in diameter and 24 inches high which covered one row foot.

Equal numbers of mated females were confined over the two varieties.

After 48 hours in the cages, the females were removed and all the stems were split. The eggs were counted and half of them were artificially placed in stems of each of the two varieties.

The aritficial introduction of the eggs was accomplished by use of a pair of 115 mm. curved dissecting forceps, a glass probe and cellulose tape. The points of the forceps were dulled so they were about 1.5mmm. in diameter. The tips of the forceps were then sharpened so that when the forceps were closed, the two tips formed a sharp blade slightly less than two millimeters in length.

These sharpened forceps were used to puncture the stem wall. When the forceps were allowed to open, the stem split vertically; usually this opening was not more than an inch long. The forceps acted as retractors and held the stem open. Through this opening the egg was passed on the tip of a finely drawn glass probe. The egg was gently touched to the inside wall of the stem to which it adhered. The probe and the forceps were then withdrawn and the incision in the stem was sealed by wrapping celiulose tape about the stem at this point. To reduce mould development a 0.5 percent sodium propionate solution was used to disinfect the instruments

and to wash the exterior of the stem prior to making the incision.

Complete records of oviposition, egg transfer and cutting was taken. Following cutting the stubs were lifted, trimmed and placed in one-ounce salve tins filled with moist soil. The tins were sealed with adhesive tape to prevent moisture loss and were held in a 5° C. temperature room for 109 days. They were then moved to a 25° C. room until emergence occurred. Before emergence began the stubs were removed from the salve tins and were placed in 21 x 70 mm. shell vials. This reduced the danger of losing adults when emergence records were taken.

EXPERIMENTAL

Oviposition

It was planned to infest plots of both varieties over a four week period beginning June 20. Infestation were to be made twice weekly, on Mondays and Wednesdays, and the transfers were to be made Wednesdays and Fridays. Owing to the slow development of the host material in the nursery it was not until July 4 that the first infestation was made.

Subsequent infestations followed July 6, 11 and 13. The females were removed and the eggs were transferred July 6, 8, 13 and 15 respectively. By July 15 all the host material,

irrespective of seeding date, was too mature for further infestation. Owing to the condition of the nursery only one half of the originally panned study was completed.

For the first infestation, individual females were placed in cages covering one row foot of the particular variety. More than 20 stems were present in each cage and since every stem had to be split and examined, recovery of the eggs was very time consuming. For subsequent infestations all but eight stems were removed from the caged plots. By reducing the number of stems available for oviposition the routine work of egg recovery was greatly reduced.

The number of females available per infestation date varied from 11 to 15 but in all 52 mated females were used. A total of 614 eggs was laid by these females; of this number 502 were manually transferred from the original oviposition site to other stems. Some eggs were lost or damaged which accounts for the discrepency in number between total laid and those transferred.

The influence of variety and infestation date on rate of oviposition by mated females is shown in Table XIII. On the average each female laid about 12 eggs during the 48-hour oviposition period. This is approximately the same mean number of eggs as was obtained in the study conducted on

TABLE XIII

Effect of variety and infestation date on number of eggs laid by mated C. cinctus females

Date of Infestation	Variety					
	Thatcher			Red Bobs		
	No. of Females	No. of Eggs Laid	Mean No. of Eggs/Female	No. of Females	No. of Eggs Laid	Mean No. of Eggs/Female
July 4	8	48	6	7	88	13
July 6	5	48	10	6	53	9
July 11	7	73	10	7	101	14
July 13	6	123	20	6	80	13
Total	26	292	11.2	26	322	12.4

bulk populations and is much higher than the mean number of eggs laid when the females were being handled individually.

Egg Transfer and Stem Cutting

The eggs were transferred from the original oviposition site to uninfested stems of the same calendar age using the technique described above. As nearly as possible the eggs laid by each female were transferred one half into each of the two varieties. The transfers were made immediately following removal of the females from the cages. In an effort to avoid environmental variations stems uniform in size and stage of development were selected.

Table XIV shows that the percentage of transferred eggs hatching was much less than is expected from normally oviposited eggs. The crudeness of the transfer methods probably contributed to these differences. There is also an indication that the environment the eggs encountered in Red Bobs stems is less suitable than in Thatcher stems. Where the eggs were transferred from Thatcher to Red Bobs or from Red Bobs to Red Bobs the percentage hatch was lower than when the reciprocal transfers from Red Bobs to Thatcher and Thatcher to Thatcher were made. The low hatch obtained was disappointing and data are inconclusive.

TABLE XIV

Hatchability of C. cinctus eggs transferred from original oviposition site

Transfer Date	Eggs laid in Thatcher and transferred to Thatcher: No. of Eggs Transferred	Thatcher: No. of Eggs Hatched	Thatcher: Percentage Hatch	Red Bobs: No. of Eggs Transferred	Red Bobs: No. of Eggs Hatched	Red Bobs: Percentage Hatch
July 6	18	7	39	24	4	16
July 8	21	9	43	24	5	21
July 13	28	6	21	29	4	14
July 15	57	15	26	56	5	9
Total	124	37	30	133	18	14

Transfer Date	Eggs laid in Red Bobs and transferred to Thatcher: No. of Eggs Transferred	Thatcher: No. of Eggs Hatched	Thatcher: Percentage Hatch	Red Bobs: No. of Eggs Transferred	Red Bobs: No. of Eggs Hatched	Red Bobs: Percentage Hatch
July 6	30	10	33	18	7	39
July 8	25	8	32	15	3	20
July 13	46	23	50	37	8	22
July 15	41	10	24	33	4	12
Total	142	51	36	103	22	21

Emergence and Sex Ratio

Emergence was first noted February 6 and emergence records were made daily until February 18. On February 21, the remaining stubs were split and the adults which had failed to emerge were sexed.

Less than 10 percent of the transferred eggs produced adults. Consequently data on the sex ratio of progeny of individual females was so limited that if offered little information. Table XV shows the summarized sex ratio data on the population developing from transferred eggs.

The sex ratio of the population developing from the eggs laid in Thatcher stems is 1.3 males per female. The comparable sex ratio for the population developing from eggs laid in Red Bobs is 0.57. Because these ratios coincide with those of field populations there is an indication that the sex ratio of a population is influenced more by the stem in which the egg was laid than by the one in which development occurred.

It is questionable if sex ratios based on less than 10 percent of the population are reliable indices of those for the whole population for it is possible that only the most viable eggs hatched and only the most vigorous individuals reached adulthood. Conclusive evidence for or against the selective fertilization hypothesis must await development of techniques favoring egg hatch and population survival.

TABLE XV

Sex of C. cinctus adults developing from transferred eggs

Eggs Laid in:	Eggs Transferred to:	Transfer Date							
		July 6		July 8		July 13		July 15	
		M	F	M	F	M	F	M	F
Thatcher	Thatcher	3	1	1	2	-	1	3	2
	Red Bobs	1	1	-	-	-	-	1	-
Red Bobs	Thatcher	-	2	1	5	3	2	2	-
	Red Bobs	2	1	-	2	-	2	-	-

Discussion

The procedures used offer a means for further study of sex determination in C. cinctus. Refinements in technique are necessary to assure adequate survival of the population but when these adjustments are made the transfer method will permit initiation of a number of studies previously impossible.

A greater percentage of transferred eggs hatched in Thatcher than in Red Bobs stems. Data are too limited to be conclusive but previous findings that Red Bobs stems provide a less suitable environment for the eggs than do Thatcher stems are supported.

SUMMARY

Throughout this work two varieties of bread wheat, Thatcher and Red Bobs, were used as host material. The parent sawfly populations were obtained near Regina, Saskatchewan.

During 1948, bulk populations were used to test the influence of a number of factors on sex determination in C. cinctus. Two of these factors, variety and mating, appeared of major importance.

Field observations that populations developed in the two varieties had widely divergent sex ratios were confirmed under controlled conditions. While the same relative trends prevailed, the actual sex ratios showed a marked shift toward maleness, the values being 2:1 and 1:1 males per female instead of the usual 1:1 and 1:2 for Thatcher and Red Bobs respectively. Thus the varietal influence on sex determination and the ultimate sex ratio is very pronounced.

Mating was the second factor which proved to be a major influence in this work. Males constituted over 97 percent of the progeny of virgin females. The origin of the small number of females in the population is not known. It is thought that some of these may have resulted from contamination of the plots by wild or escaped females. However, supplementary work

the same summer, wherein the progeny of individual females was studied, showed conclusively that females could be produced uniparentally.

The data indicate that a lowerepercentage of the progeny of virgin females cut the stems. It is suggested that the male has less resistance to adverse environmental conditions. Hence development is retarded or death occurs depending upon the degree of environmental resistance.

Generally Red Bobs is cut less severely than Thatcher which indicates higher mortality in the variety. Likewise sawfly populations developing in Red Bobs stems show a preponderance of females. It is proposed that these two observations are related through the mortality occurring primarily in the males.

During 1948 an effort was also made to compare the progeny of individual females under rigidly controlled conditions. Difficulty was encountered in securing oviposition and owing to the small proportion of the eggs laid it was impossible to gain a true comparison of the sex ratios of the progeny.

In 1949 a technique was used whereby the eggs were manually transferred from the original oviposition site to other stems. By this procedure it was possible to test the hypothesis that selective fertilization of the eggs during oviposition accounted for the diverse sex ratios of populations developing in different varieties. In this work a further

indication was gained that Red Bobs stems offer a less suitable environment for the eggs than do Thatcher stems; a lower percentage of the eggs developed following transfer to Red Bobs stems than to Thatcher stems.

Owing to the limited number of progeny, conclusive evidence on the selective fertilization hypothesis is lacking. There is an indication, however, that the original host variety has a bearing on the ultimate sex ratio of the population.

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