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

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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ARTHUR JAMES McGINNIS

A THESIS

Submitted to the Graduate Committee

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at

Montana State College

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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 beelte 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 thelyotokus 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 determiningg 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

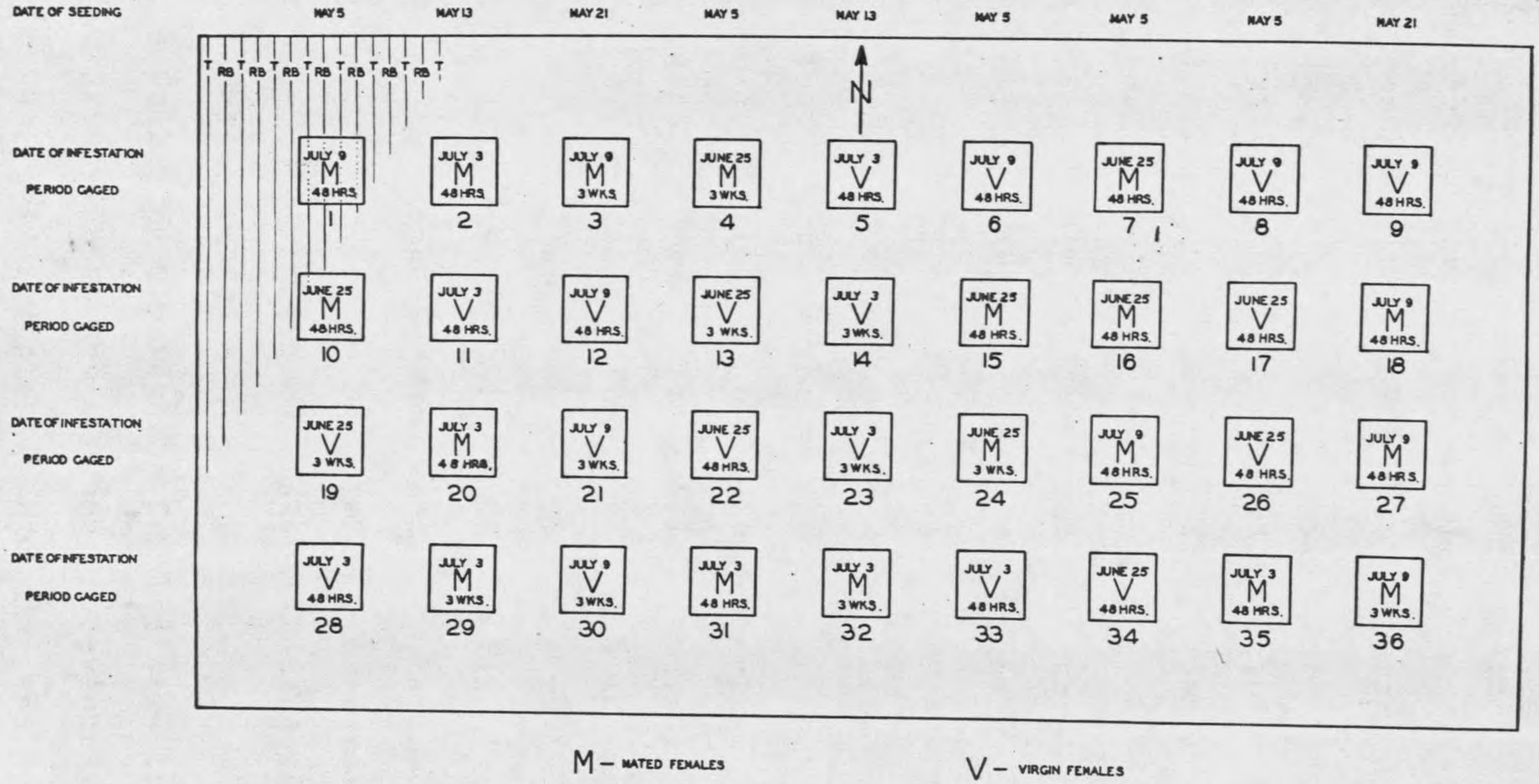

Figure 1 - Plot Design

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.