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E. L. Nieman

G. R. Manglitz

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E. L. Neiman

G. R. Manglitz

University of Nebraska – Lincoln College of Agriculture
The Agricultural Experiment Station
E. F. Frolik, Dean; H. W. Ottoson, Director

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SUMMARY

These studies indicate that smooth bromegrass, *Bromus inermis* Leyss, is the only host of the bromegrass seed midge, *Stenodiplosis bromicola* Marikovskiy and Agafonova, in Nebraska. The midge did not infest two common annual brome species under field conditions.

The bromegrass seed midge was observed to overwinter in the field as diapausing larvae in fallen florets. Diapausing larvae were able to survive the harvesting process and at least one year of seed storage. A preconditioning cold period and available free moisture during the emergence period were necessary to break larval diapause. Adults of the overwintering generation normally emerged in late April or early May.

Larvae of the first and second summer generations fed upon unpollinated bromegrass florets and completed development without diapause. Adults of the first summer generation emerged during the first half of May; those of the second emerged during the last half of May. A summer generation of the midge took 14 to 18 days to develop.

Pollination of the bromegrass plants occurred at the end of May or the beginning of June. Progeny of the second summer generation adults developed in pollinated florets and entered diapause to form the overwintering generation. Three generations were observed per year. Studies indicated that the female midges showed some preference for unpollinated florets as ovipositional sites.

Damage to smooth bromegrass florets caused by feeding of midge larvae was negligible in some areas of Nebraska in some years. However, infestation rates of over 50% were observed in other years. Shattering of infested florets from the spikelets was observed during the period when florets were infested by diapausing larvae. This may have resulted in a substantial additional loss of seed because uninfested seed was also lost in this process.

The midge has been collected from all counties surveyed in Nebraska and seems to be widely distributed throughout the midwestern states.

An apparently undescribed species of *Tetrastichus* (Hymenoptera: Eulophidae) was observed to parasitize all stages of the bromegrass seed midge. Parasitism rates greater than 90% were observed. The parasite larvae do not kill the midge until damage to the bromegrass floret has occurred. However, a high parasite population may help to reduce the population of the bromegrass seed midge in succeeding generations.

The Biology and Ecology of the Bromegrass Seed Midge in Nebraska

Elray L. Neiman and George R. Manglitz[1]

INTRODUCTION AND LITERATURE REVIEW

In the spring of 1965 we found a small midge developing within the seed heads of smooth bromegrass *(Bromus inermis* Leyss.) in the vicinity of Lincoln, Nebraska. This midge was subsequently identified as *Stenodiplosis bromicola* Marikovskiy and Agafonova (4) and is now commonly called bromegrass seed midge. Prior to that time this insect had been positively identified only in Russia where it was first described as a species in 1961 (Marikovskiy and Agafonova (8). However, an unidentified midge, quite likely the same species, has been reported as being widely distributed, since 1948, in the United States (Wisconsin, North Dakota, Indiana and Oklahoma) and at times quite damaging to the production of smooth bromegrass seed (Nielsen and Burks (9).

One or two species of parasitic Hymenoptera appear to always be associated with the midge in bromegrass heads wherever the midge occurs (1,9). These parasites leave visible evidence of their presence, in contrast to the midge which does not, by making tiny exit holes in the florets where they have developed by feeding upon midge larvae. This may be the reason why there are erroneous reports in which the hymenopterous parasites have been identified as the cause of seed failure in bromegrass (1,3).

Agafonova (2) reported on the biology of the midge as it relates to two species of bromegrass grown in Russia, where the midge is widely distributed. It has been found in the provinces of Kursk, Voronezh, Orlovek, Ryazan, Moscow, Leningrad and Kustenay and in the Krasnodar Territory. In Russia the midge attacks only three species of grass *(Bromus inermis, B. erectus and B. riparius),* However,

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Graduate Research Assistant, Department of Entomology. Research Entomologist, Entomology Research Division, ARS-USDA, and Associate Professor, Department of Entomology. Adapted from a portion of a thesis, by the senior author, presented to the Graduate faculty of the University of Nebraska in partial fulfillment of the requirements for the Degree of Master of Science. This study was supported by the Agricultural Research Service of the U.S. Dept. of Agriculture through Cooperative Agreement No. 12-14-100-8461(33) with the Nebraska Agricultural Experiment Station.

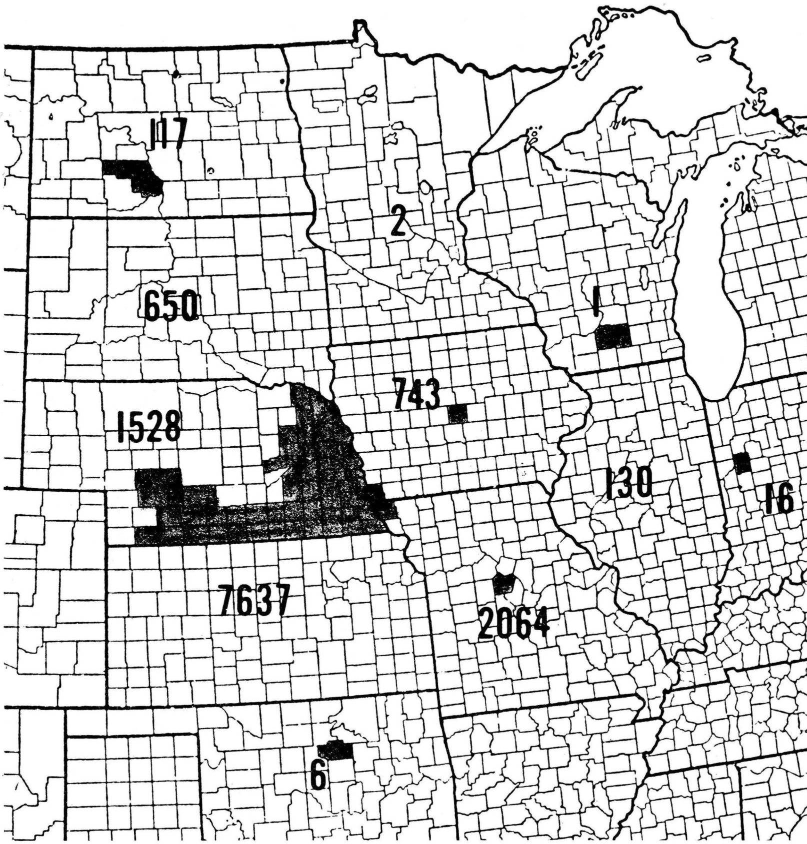

Figure 1. Production of smooth bromegrass seed and distribution of bromegrass grass seed midges by states. Figures within state boundaries indicate 1964 seed production in thousands of pounds (U.S. Bureau of the Census (11). *Stenodiplosis bromicola* has been identified in Nebraska, Iowa and Missouri (shaded areas). In North Dakota, Oklahoma, Wisconsin, and Illinois (shaded areas) midge infestations (species not identified) have been reported by Nielsen and Burks (9).

its greatest economic damage appears to occur in *B. inermis* (smooth bromegrass), with infestation rates reaching 100% in some years.

Smooth bromegrass seed production in the north central United States occurs in the general area where the bromegrass seed midge is either known to occur or is suspected of occurring (Figure 1).

This study was initiated because of the possible threat by the midge to the production of smooth bromegrass seed in Nebraska and adjacent states. The purpose of this study was to learn more of the biology and ecology of *S. bromicola* as it occurs in Nebraska. More specifically, studies were conducted to determine the midge's host range; its distribution and the nature and extent of its damage to the developing seed of smooth bromegrass; the seasonal occurrence of the midge, its life cycle, number of generations per year, overwintering stages and the nature of its diapause. Studies were also made upon the parasites of the midge as they affected occurrence and populations of the midge.

BIOLOGY OF THE MIDGE

The study of the biology of the bromegrass seed midge consisted of investigations of the host range of the insects, of the overwintering stages and conditions necessary to break diapause and of the mechanics of adult emergence and oviposition. Also studied were larval feeding habits, pupation sites, causes for diapause initiation, seasonal occurrence of immature and adult stages of the midge and distribution of the immature population within the brome panicle.

Host range studies were conducted to determine if the midge also infests two species of annual bromegrass commonly and widely distributed in Nebraska. These two species, downy brome (*Bromus tectorum* L.) and hairy chess (*Bromus commutatus* Schrad.), are considered weedy grasses (6) that at times present a problem in certified fields of smooth bromegrass.

In 1966 and 1967 studies were conducted to investigate the biological characteristics of the midge in Nebraska. Agafonova (2) reported on the biology of the midge in the Kursk Province of Russia. Thus a comparison could me made between the biology of the midge in Russia and Nebraska.

Methods

Host range investigations were made on field-collected panicles of downy brome and hairy chess which were brought into the laboratory, caged and observed for midge emergence. Floret samples were randomly taken from the panicles and examined under a microscope. These observations were made before pollination, after pollination and during caryopsis formation. Midges were also confined in cages on panicles of both species to determine whether oviposition would occur.

Samples of soil and trash from an unharvested smooth bromegrass field were collected and examined for the presence of the overwintering stage of the midge. Certified smooth bromegrass seed samples were examined to learn if the midge could survive harvest and storage.

Experiments were conducted to define the general conditions necessary to break the diapause of the overwintering stage of the midge. A field-collected sample of infested florets was divided into eight lots of seed, half of which were treated at 40° F for five months. At the end of the treatment period the temperature was raised to 75° F. The other four lots of seed were maintained at 75° F for the entire experimental period. Two of the four lots of seed in each treatment were soaked in water while the other two lots of seed were maintained in a dry condition. One lot of seed of each temperature-moisture treatment combination was placed in an environment in which the relative humidity was maintained at 50% while the other lot of seed was kept in an environment of 100% relative humidity. Regulation of the relative humidity was accomplished by placing the florets into desiccators in which the relative humidity was regulated by sulphuric acid solutions (7).

Smooth bromegrass florets were collected from the field at frequent intervals and examined to determine the stage of midge present in florets. These observations also indicated the seasonal development of immature stages of the midge.

Distribution of immature population of the midge within the bromegrass panicle was studied by dissecting florets randomly taken from the upper and lower halves of the panicle. These observations were also made to determine if the female midges exhibited an ovipositional preference for florets in various stages of development.

Sweep net samples were taken from the field to assess changes in the population of adult midges during the season. This information was used to estimate the number of generations per year.

Results and Discussion

Host Range

Midges or their parasites were not observed emerging from caged panicles of either downy brome or hairy chess. Microscopic examination of the florets showed no evidence of midge infestation. Attempts to rear the midge on caged panicles in the greenhouse failed. Oviposition was not observed in the field.

While there is evidence that the bromegrass seed midge does infest several species of bromegrass other than smooth bromegrass (2,9), all of these species are perennial plants that are not commonly found in Nebraska. Apparently downy brome and hairy chess do not serve as host plants under field conditions in Nebraska. This may be an effect of the stage of maturity reached by the annual bromes by the time the adult midges of the overwintering generation first emerge. At this time the caryopses of the annual species have been formed and are ripening.

Overwintering and Breaking of Diapause

Three samples of bromegrass sod were taken from an unharvested

Table 1. Infestation rates and germination percentages of five lots of certified smooth bromegrass seed. Examined winter 1967.

Variety	County of origin	Date harvested	Percentage of seed: Germinated	Percentage of seed: Infested
Lincoln	Lancaster	1966	67.0	2.5
Lincoln	Seward	1966	47.5	3.0
Lincoln	Nance	1966	36.0	4.0
Sac	Madison	1966	42.5	3.0
Lyon	Lancaster	1965	78.0	1.5
		Mean	54.2	2.8
				r = -0.94*

* Significant at P = 0.05.

field in Saunders County in September 1966. Examination of the samples showed an average of three non-diapausing midge larvae per square foot of sod. The diapausing larvae were found in shattered florets among the trash on the soil surface.

Examination of certified brome seed samples showed that diapausing larvae can survive the harvesting process and at least one year of seed storage (Table 1). Agafonova (2) reported that larvae were found in samples of seed up to two years after harvest. The infestation rates ranged from 1.5% to 4.0% with a mean of 2.8%. The actual infestation rate was probably higher than the observed rate since florets attacked late in the developmental period may not have been prevented from germinating. The germination percentage varied inversely with the infestation rate (r = 0.94) indicating that the presence of diapausing larvae probably affected the germination percentage along with being a source of infestation of new seedings of smooth brome.

Results of the laboratory experiments conducted to define the general temperature and moisture requirements necessary to break larval diapause are summarized in Table 2. Diapause was broken and adult emergence occurred only when the florets were preconditioned at 40° F (4.44° C) and when free moisture was present during development. A greater number of adult midges emerged from florets main-

Table 2. Effect of preconditioning dispausing larvae on midge emergence. Two temperatures (40° and 75°F) and differences in free moisture (present or absent) were used with two relative humidities (50% and 100%) during the period of development at a temperature of 75°F.

Free moisture	Relative humidity (%)	Midge emergence (number), at preconditioning temp. (°F) of: 40°F	75°F
Present	50	57	0
	100	209	0
Absent	50	0	0
	100	0	0

Table 3. Time of emergence (days after preconditioning period) and sex of emerged adults.

Time after preconditioning (days)	Midge emergence (sex and number)[a]		
	Males	Females	Total
10	0	0	0
11	4	2	6
12	9	3	12
13	7	11	18
14	11	16	27
15	20	14	34
16	23	15	38
17	14	16	30
18	9	9	18
19	7	10	17
20	2	2	4
21	0	2	2
22	2	0	2
23	1	0	1
24	0	0	0

[a] Chi-square = 0.624 (not significant at P = 0.05) indicating an apparent fit to a 1 : 1 ratio.

tained at 100% relative humidity (209 midges) than from florets maintained at 50% relative humidity (57 midges). The requirement for free moisture (rain or dew) during the development of some species of cecidomyiids has been noted by Barnes (5) and Walter (12).

Adult Emergence and Oviposition

Emergence of adult midges in the previous experiment, that was designed to break larval diapause, indicated that emergence started 11 days after the end of the preconditioning period (Table 3). The number of adults emerging per day increased daily until the 16th day and then declined. No emergence was observed after the 23rd day.

The sex ratio of the emerged adults was nearly 1:1 (109 males to 100 females, not significant P = 0.05). Males generally emerged earlier in the day than did the females. The tiny delicate adults are shown in Figure 2. Observations made by Agafonova (2) indicate that emergence of the over-wintering generation normally coincides with the appearance of the first panicles of the earlier maturing *B. riparius* plants.

Later, as panicles of smooth bromegrass emerge from the boot, the midge shifts its attack to those plants. In field observations in Nebraska we have been unable to capture adult midges before the emergence of the first panicles of smooth bromegrass.

Mating occurs soon after emergence and oviposition begins immediately if suitable oviposition sites are found. During the egg laying process the female midge aligns herself facing the tip of the floret. The

Figure 2. Adults of the bromegrass seed midge, *Stenodiplosis bromicola.* Male left, female right. (10 X)

ovipositor is forced between the lemma and palea of the floret and one (rarely 2 or 3) eggs are laid.

Few eggs were observed within the florets because of their small size (0.5 mm long by 0.1 mm wide) and the difficulty encountered in finding and observing them without displacing the egg from the floret. Those eggs that were observed were attached to the inner side of the palea about 2 to 3 mm from the tip (Figure 3). Dissection of newly emerged female midges showed that the ovaries were fully developed at the time of emergence and each female contained from 80 to 100 eggs. The eggs are elliptical with one end rounded and the other end drawn into a filament slightly shorter than the egg. Agafonova (2) reported that the egg stage lasts from 2 to 3 days.

Habits of Larvae and Pupae

Newly hatched larvae move down the floret between the lemma and palea to the developing ovary. The larva feeds with its head toward the base of the floret, withdrawing the contents of the ovary until all that remains is the shriveled ovary wall. Normally, only one larva was observed per floret. When hatched the larvae are colorless but change to a bright yellow when full-grown at 1.5 to 2.0 mm (Figure 4). Larval feeding completely destroys the ovary, consequently no seed is set. The development of stamens is stopped, florets do not open and no pollen is shed from infested florets.

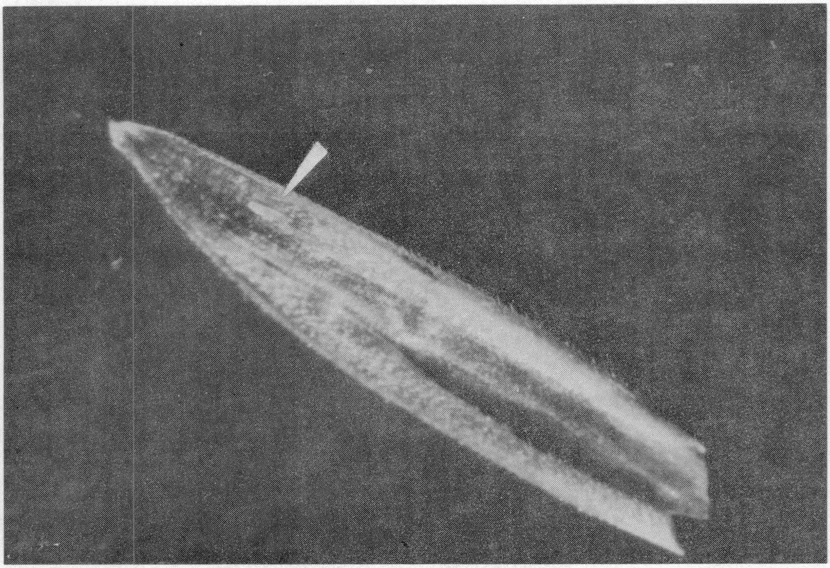

Figure 3. Photograph of *S. bromicola egg* attached to the inner side of an excised palea of a brome floret approximately 2mm from the tip of the palea. Magnified about 10 X.

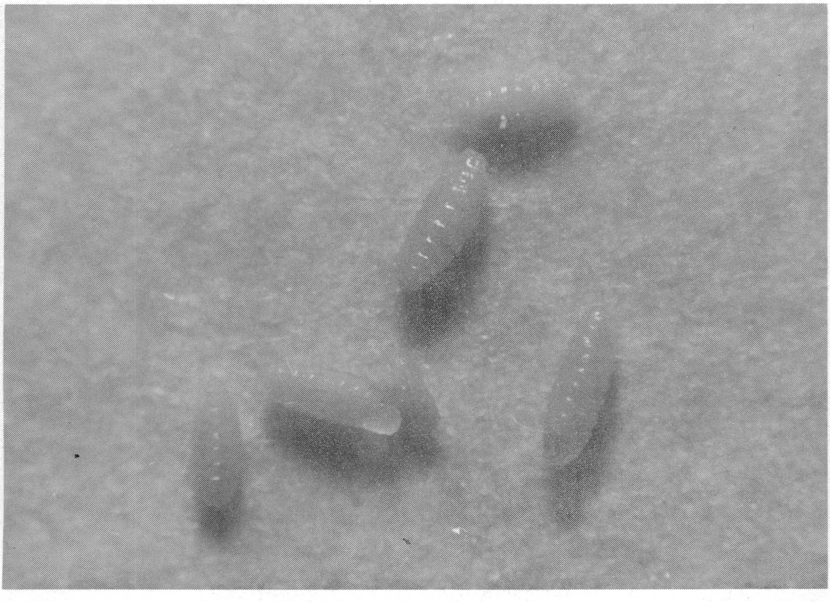

Figure 4. Photograph of mature non-diapausing lravae of *S. bromicola.* Magnified about 10 X.

Figure 5. Photograph of *S. bromicola* pupa. Magnified about 30 X.

Agafonova (2) reported that the larval stage lasts from 6-9 days depending upon the conditions prevailing during the period of larval development. At maturity, the larvae reverse their position within the floret and pupate at the feeding site.

The pupa's appendages are enclosed in a sclerotized membrane that first is yellow, later darkening to brown or black. The abdomen is yellow and is enclosed in a transparent membrane, reported by Agafonova (2) to be the larval "skin" of the last instar (Figure 5).

The pupa can move short distances by flexing its abdomen. In this manner the pupa moves to the tip of the floret where it fastens itself by the posterior end of the abdomen. Reports by Agafonova (2) indicate that the pupal stage lasts from 2 to 3 days. After emergence, the pupal case may remain attached to the tip of the floret but is easily dislodged by wind and rain.

If the pupal case dislodges, after adult emergence, no evidence of midge infestation remains and it is nearly impossible to determine whether an individual floret was destroyed by a midge larva or whether floret development failed.

Initiation of Diapause

Larval development of the midge, as previously described, is altered when the larva feeds in pollinated florets with developing caryopses.

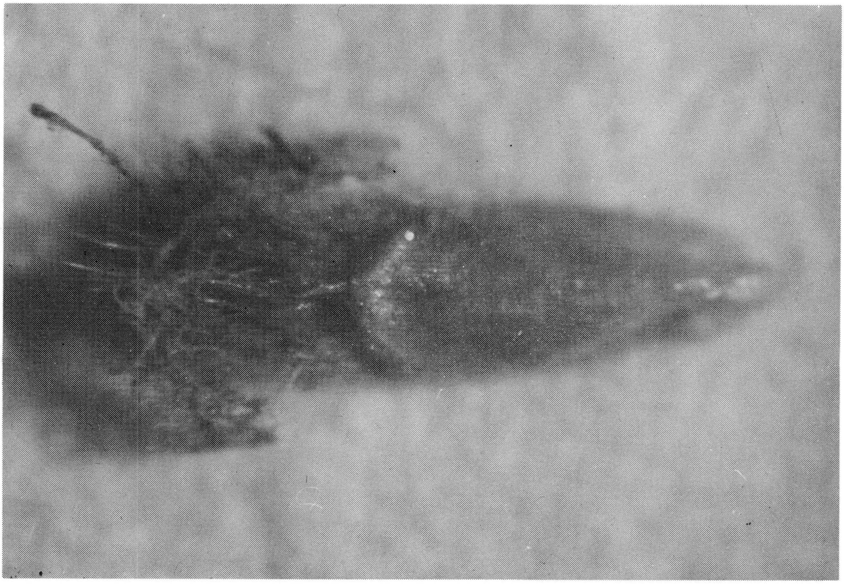

Figure 6. Photograph of a cocoon containing a diapausing larva of *S. bromicola* attached to the remains of a bromegrass caryopsis. Magnified about 20 X.

Then, instead of pupating, the larva spins a cocoon at the feeding site and enters diapause.

The cocoon resembles the developing caryopses and is attached near the base of the floret (Figure 6). Diapause occurs only when larvae feed upon the fertilized ovary or developing caryopses of the floret. Larval diapause has never been observed to occur prior to the time the host plants begin pollinating.

Thus it appears that the initiation of diapause is controlled by the larval diet (unfertilized ovaries versus fertilized ovaries). Other factors, such as light, temperature and moisture, may affect the period of plant fertilization. Agafonova (2) reported that in Russia, the midge had two generations during a warm dry year, which tended to speed the development of the brome plants, but in a cool wet year (conditions which prolonged plant development) the midge had four generations.

Seasonal Occurrence and Distribution of Immature Stages Within Panicles

Smooth bromegrass panicles were collected at frequent intervals during the 1967 season and examined to determine the seasonal development of the immature stages of *S. bromicola*. Collections were made twice weekly from June 2 to June 30.

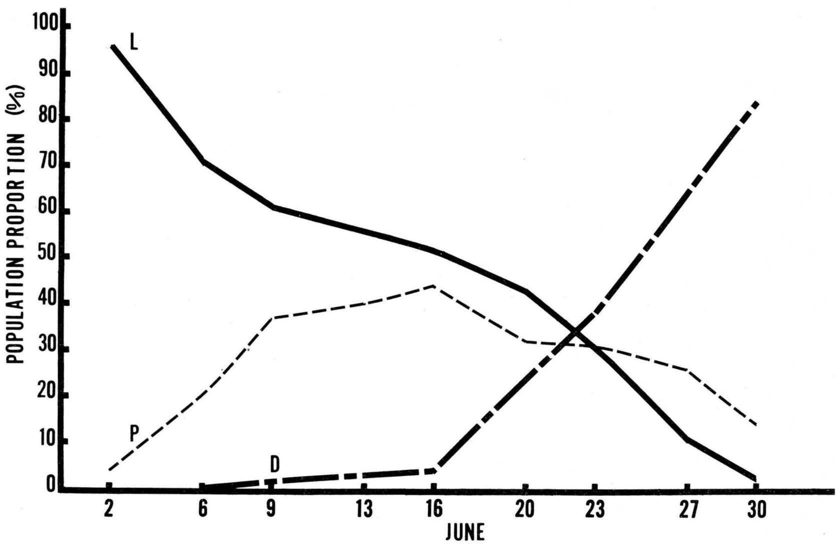

Figure 7. Graphic representation of the changes that occurred in the larval (L), pupal (P) and diapausing (D) populations of the bromegrass seed midge between June 2 and June 30, 1967. Each population survey is shown as the proportion (%) of the total immature population.

Examination of the resulting population curves (Figure 7) shows that the proportion of the immature population in the larval stage decreased rapidly from June 2 to June 9 while the proportion of the population in the pupal stage increased. From June 9 to June 16 the larval population continued to decrease and the pupal population continued to increase but both populations changed more slowly.

Diapausing larvae were first observed on June 9 but represented only a small proportion of the immature population. Observations made from June 16 to June 30 showed a rapid increase in the proportion of larvae undergoing diapause. This rapid increase of diapausing larvae occurred about 14 days after the initiation of pollination of the bromegrass plants from which the floret samples were taken. The 14-day period represented the time required for the larvae to grow and spin a cocoon.

Development of the reproductive organs of smooth bromegrass plants is generally prolonged over a period of time. Spikelet formation starts at the top of the panicle and progresses downward. Caryopses in the upper part of the panicle may mature up to seven days earlier than those in the lower portion. Field-collected smooth bromegrass panicles were examined to determine if female midges exhibited a preference for florets in a certain stage of maturity as ovipositional sites. This was done by assessing the larval, pupal and diapausing populations in the upper and lower levels of the brome panicle on six dates from June 6 to June 23, 1967 (Table 4). The proportion of larvae in the lower half of the panicle was consistently higher than that found in the upper half.

The proportion of larvae in each level of the panicle remained relatively constant until June 23 when more larvae were found in the lower, more immature portion of the panicle. Since the majority of the larvae were observed to be infesting florets in the lower portion of the panicle, it was concluded that female midges showed preference for immature florets.

Distribution of pupae within the panicle showed more variation between observations than did larval distribution. Generally, except for the June 6 observation, more pupae were found in the upper level of the panicle.

A possible explanation is that the pupae observed during this period were derived from eggs laid in the upper portion of the panicle 14 to 18 days earlier. At that time the florets in the upper level were in an early stage of maturity whereas the florets in the lower level were less fully developed and not yet suitable as ovipositional sites.

Since pollination of the bromegrass plants from which the floret samples were taken did not start until the end of May or the beginning of June, no mature diapausing larvae were found during the first half of the observation period. About 14 days after pollination was first observed in the upper level of the panicle the majority of the diapausing larvae were also found in the upper level. Fourteen days after, when pollination had progressed to the middle of the panicle, nearly equal numbers of diapausing larvae were found at each level.

Table 4. Percentages of the total larval, pupal and diapausing populations found in the upper and lower levels of the bromegrass panicle on each of 6 dates from June 6 to June 23, 1967.

Date	Panicle level	Percentage of population of: Larvae	Pupae	Diapausing larvae	Total
June 6	lower	51.7	56.8	0.0[a]	53.2
	upper	48.3	43.2	0.0	46.8
June 9	lower	56.9	44.3	0.0	52.2
	upper	43.1	55.7	0.0	47.8
June 13	lower	54.8	47.4	0.0	43.5
	upper	45.2	52.6	0.0	56.5
June 16	lower	56.2	41.2	37.5	48.9
	upper	43.8	58.8	62.5	51.1
June 20	lower	50.0	73.5	47.5	45.2
	upper	50.0	62.5	52.5	54.8
June 23	lower	60.0	40.6	56.5	53.4
	upper	40.0	59.4	43.5	46.6

[a] No diapausing larvae were found in samples collected on June 6, 9 and 13.

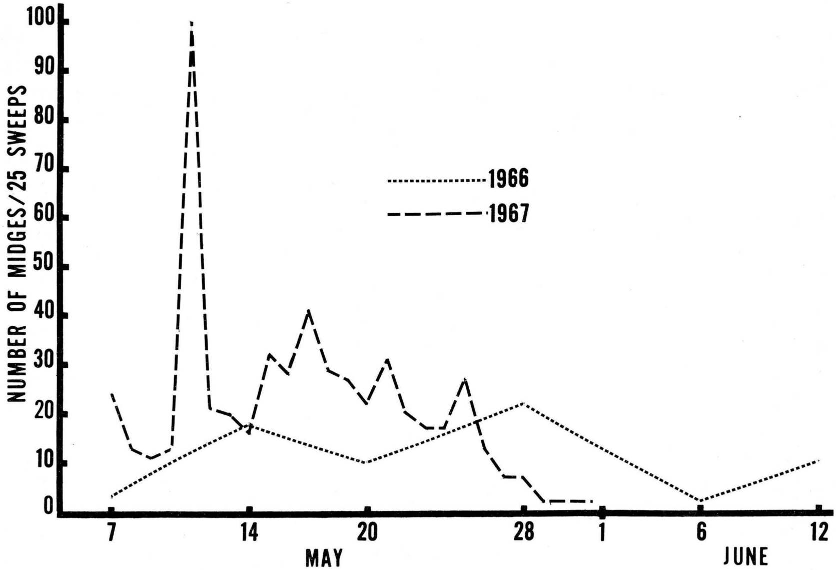

Figure 8. Emergence curves determined by sweep net catches of bromegrass seed midge adults. Samples taken between May 7 and June 12, 1966 and May 7 and May 31, 1967.

Observations made on other samples of florets, collected after caryopsis formation was complete, showed that the diapausing generation of the midge was nearly equally distributed throughout the panicle.

Seasonal Occurrence of the Adult

Sweep net samples were taken during the 1966 and 1967 seasons to determine adult emergence in the field. From these data (Figure 8) the number of generations per year was estimated. In 1966, samples were taken at weekly intervals from May 7 to June 12. Daily samples were taken in 1967 from May 7 to May 31. Emergence peaks in 1966 occurred on May 14, the peak emergence of the adults of the first summer generation. Another peak was probably reached on or after June 12 and consisted of adults of the second summer generation. The progeny of the second summer generation adults developed in pollinated florets and entered a state of diapause.

Panicles of smooth bromegrass appeared earlier in Nebraska in 1967 than in 1966 and the emergence peak of adults of the overwintering generation and the first summer generation were not determined. Peak emergence of the first summer generation occurred on May 11. Weather conditions during the latter half of May were characterized by subnormal temperatures and abnormal amounts of rainfall with the result that adult emergence occurred sporadically from May 15 to May 25. The adults collected during this period were thought to be derived, primarily, from the second summer generation, although some individuals of the first summer generation may have constituted a portion of the emerging population early in the period. Since development of bromegrass plants was prolonged because of cool temperatures, considerable overlapping of generations probably occurred.

From observations made in Nebraska it was concluded that the seasonal cycle of *S. bromicola* did not differ substantially from that reported by Agafonova in the Kursk Province of Russia. Bromegrass seed heads and the midge both appeared earlier in the season in Nebraska than in the Kursk Province. However, this is probably due to climatic conditions since the Kursk Province is 11 degrees farther north than is Nebraska.

PARASITES OF THE MIDGE

During the course of the studies on biology of the bromegrass seed midge a parasite *Tetrastichus* sp (Hymenoptera: Eulophidae) was found to be associated with the midge.

Methods

Most of the parasite observations were made at the same time as, and in conjunction with, observations on the midge. Therefore, while making floret dissections and observations of the midge, it was possible to determine the stage of the midge attacked by the parasite and to estimate the effectiveness of the parasite.

Field-collected smooth bromegrass panicles were caged and the emergence of the adult parasites was observed in relation to the emergence of the midge adults.

Attempts were made to rear the parasites upon the stage of the midge with which it was found associated in order to obtain positive host determination. This was done by caging adult parasites with florets known to be infested by the stage of midge serving as a host for the parasite. The florets were caged until the adult parasites emerged or until an examination of the florets showed no immature stages of the parasite to be present.

Results and Discussion

The examination of florets infested by the non-diapausing stages of the midge showed parasite larvae feeding upon both the larvae and pupae of the bromegrass seed midge. Adult parasites that emerged from infested panicles were identified by B. D. Burks (see acknowledgments) as an apparently undescribed species of *Tetrastichus* (Hymenoptera: Eulophidae).

Dr. Burks reported that the species had always been reported in association with bromegrass seed and that a host determination had not been made (personal communication).

Agafonova (2) reported that a species of *Tetrastichus* parasitized the midge pupae in Russia.

Ahring (3) reported that a *Tetrastichus* species caused damage amounting to 30% of the florets of Southland smooth bromegrass in Oklahoma. It is probable that this species is the same as that found parasitizing the midge in Russia and Nebraska and is not the primary cause of damage to smooth bromegrass in Oklahoma.

Diapausing midge larvae have also been observed to be parasitized by this species. Parasitism of the diapausing larvae was observed in samples collected late in July and also in trash samples collected from an unharvested bromegrass field in September.

An examination of samples of smooth bromegrass sod collected from an unharvested field in Saunders County, Nebraska, in September 1966 showed that 180 of 190 diapausing midge larvae were parasitized by *Tetrastichus* larvae. Adult parasites reared from these samples were, curiously, nearly all males.

Results of the examination of florets collected from June 6 to June 23, 1967 are shown in Table 5. Only one parasite larva was observed to feed, externally, on an individual midge larva or pupa. The rate of parasitism of *Tetrastichus* affecting the non-diapausing midge larvae ranged from 39.7% to 94.8% with a mean of 73.4% for the entire observation period. The parasitism rate for midge pupae increased from 64.4% to 100% as the period progressed. The mean parasitism rate for *Tetrastichus* attacking pupae was higher than the

Table 5. Parasitism of immature stages of bromegrass seed midge by *Tetrastichus* sp. on six dates from June 6 to June 23, 1967.

	Tetrastichus sp. in:					
	Larvae		Pupae		Diapausing larvae	
Date	No.[a]	%	No.[a]	%	No.[a]	%
June 6	132/144	91.7	38/59	64.4	0/0	......
June 9	55/58	94.8	34/35	97.1	0/0	......
June 13	29/73	39.7	18/19	94.7	0/0	......
June 16	24/48	58.3	40/40	100.0	0/4	0.0
June 20	17/36	47.2	28/28	100.0	1/20	5.0
June 23	17/20	85.0	16/16	100.0	9/23	39.1
	278/379	73.4	174/197	88.3	10/49	20.4

[a] Number of parasites/number of hosts.

mean parasitism rate for larvae possibly indicating a slight pupal preference. Most of the parasites were in the pupal stage at the end of the observation period. This may indicate that the winter is spent in that stage.

A small number of diapausing midge larvae were found to be parasitized near the end of the observation period. The examination of a sample of florets collected from the same location in the latter part of July 1967 showed 70.6% of the diapausing midge larvae to be parasitized.

To positively determine the host of the *Tetrastichus* species, adult parasites were caged on florets infested by non-diapausing midge larvae. Ten florets containing the larvae were caged with 20 *Tetrastichus* adults. The female parasites were observed to oviposit by penetrating the lemma or palea of the brome floret. No attempt was made to recover the eggs of the parasites because of the limited number of florets used in the study. The adult parasites were removed after three days and the florets were maintained at room temperature for the remainder of the observation period. Three weeks later three adult parasites had emerged from the bromegrass florets, indicating that the life cycle of the parasite is somewhat less than 21 days. The three parasites were identified by Burks as *Tetrastichus* sp.

DISTRIBUTION AND DAMAGE

Studies were conducted to determine the distribution of and the damage caused by the midge in Nebraska. The search for the midge was concentrated in the same geographical area where smooth bromegrass seed production is concentrated (east and south). Limited information on the distribution of the midge outside Nebraska was obtained by the authors personally and by communication with other researchers.

Methods

Three general methods of detecting midge infestations were used. The first involved taking sweep net samples in bromegrass fields and counting the number of adult midges captured. The second involved the microscopic examination of commercially harvested seed samples. Because the seed samples contained only diapausing larvae and very few damaged florets neither of these first two methods produced information on amount of damage done by the midge.

The third method involved the collecting of panicles and examining a portion of the florets for the presence of immature stages of the midge. Besides locating infestations, this method also produced information on the amount of damage done by the midge. This latter

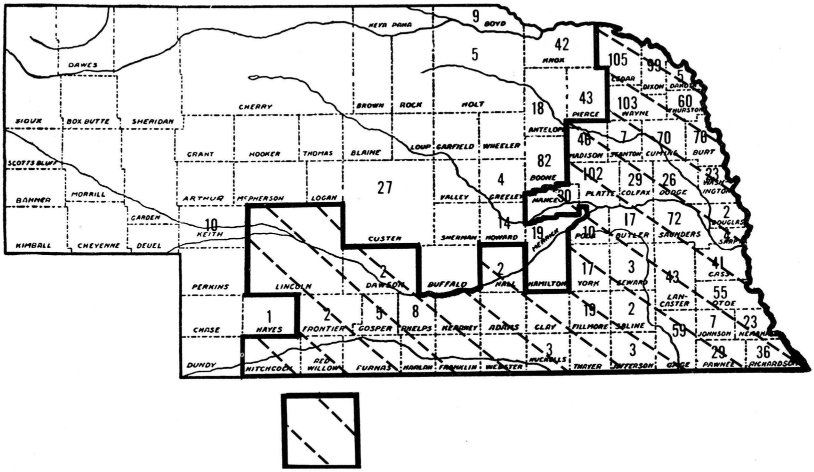

Figure 9. Distribution of bromegrass seed midge in Nebraska. Numbers refer to smooth bromegrass seed production in thousands of pounds.

method was used during the surveys made in early July 1965 (southern area) and late June 1966 (southeastern). Each sample consisted of all florets from five spikelets randomly selected from each 10 panicles. Each floret was placed in one of the following categories: normal, in which a normal ovary or caryopsis was found; undeveloped, no caryopsis was forming but there was no evidence of midge infestation; or infested; diapausing larvae found in the floret.

Results and Discussion

Distribution

S. bromicola was found in every county searched. The locations of the 49 Nebraska counties where infestations were found are shown in Figure 9. This figure also includes bromegrass seed production for 1964 given in thousands of pounds for each Nebraska county (U.S. Bureau of the Census (10)). Because of the ease with which midge infestations were found it seems within reason to speculate that the midge may be found wherever bromegrass is grown.

States where infestations of *S. bromicola* are either known or suspected are shown in Figure 1. Records for states other than Nebraska were obtained from several sources. The records for Fremont County, Iowa, and Atchison County, Missouri, were made by the authors. The presence of the midge in Story County, Iowa, was determined by J. L. Jarvis (personal communication). The record for Cooper County, Missouri, was obtained by L. Peters and G. W. Thomas (personal communication). These are the only verified county records outside of Nebraska where positive identification of the midge has been made.

The records for Oklahoma (Payne County), North Dakota (Morton County), Wisconsin (Dane County) and Indiana (Tippecanoe County) were derived from damage reports by Nielsen and Burks (9) and Ahring (3). Though positive identification of the midge has not been made in these counties, it is probable that the damage reported was caused by *S. bromicola*.

Damage

At the time (late June and early July) samples for damage evaluation were taken the midge larvae had entered diapause. This diapausing stage was the only one found in the florets examined. The damage caused by previous generations of the midge was difficult to determine because all evidence of the midges' presence was lost when the shed pupal skins were dislodged from their location near the tip of the floret. With direct evidence of infestation gone it was not possible to determine whether failure of an ovary to develop resulted from midge feeding or from some other cause. Thus, the observed infestation rates, of diapausing larvae only, were low estimates of the total infestation during the season.

Samples of smooth bromegrass panicles collected in the 1965 survey and examined in the laboratory showed infestation rates ranging from 0.0% to 11.4% (Table 6). The Phelps County sample was the only one that showed no evidence of infestation. The highest rate was determined for the Lancaster County sample. The average infestation rate for all samples was 3.0%.

The highest percentage of undeveloped florets (83.2%) was found in the Kearney County sample which also had the lowest percentage of normal florets (16.2%). The sample from Red Willow County which showed the lowest percentage of undeveloped florets (34.5%) also showed the highest percentage of normal florets (63.7%).

Infestation rates of the samples collected in 1966 ranged from 4.5% in Cass County sample No. 3 to 50.4% in Cass County sample No. 4 (Table 6). The average infestation rate for all samples was 26.1%; considerably higher than in 1965. Sample No. 4 from Cass County also showed the lowest percentage of undeveloped florets (24.8%) while the highest percentage (62.6%) was found in Gage County sample No. 1.

The average percentage of undeveloped florets for samples was 39.4%. The percentage of normal florets ranged from 21.7% in Lancaster County sample No. 2 to 54.5% in the sample collected from

Table 6. Percentage of normal, undeveloped and infested florets in field-collected smooth bromegrass samples. 1965 samples were collected on July 3 from 11 southern Nebraska counties, 1966 samples were collected on June 30 in southeastern Nebraska, southwestern Iowa and northwestern Missouri.

County[a]	Number of florets examined	Normal	Percentage of florets	
			Undeveloped[b]	Infested[c]
1965				
Lancaster	264	18.2	70.4	11.4
Seward	254	33.1	62.6	4.3
Saline	296	48.0	48.6	3.4
Fillmore	309	29.5	67.3	3.2
Clay	303	52.5	41.2	6.3
Adams	293	39.3	59.7	1.0
Kearney	316	16.2	83.2	0.6
Phelps	412	51.7	48.3	0.0
Harlan	346	56.3	43.1	0.6
Furnas	338	44.7	54.4	0.9
Red Willow	339	63.7	34.5	1.8
Mean (1965)	316	41.2	55.8	3.0
1966				
Lancaster #1	333	27.6	41.4	31.0
Lancaster #2	226	21.7	44.7	33.6
Gage #1	270	31.1	62.6	6.3
Gage #2	224	42.0	35.2	22.7
Cass #1	299	27.1	53.8	19.1
Cass #2	274	27.7	28.1	44.2
Cass #3	247	51.4	44.1	4.5
Cass #4	266	24.8	24.8	50.4
Fremont	255	54.5	25.5	20.0
Atchinson	334	40.1	44.6	15.3
Mean (1966)	273	34.5	39.4	26.1

[a] Fremont sample from Iowa, Atchison sample from Missouri and all other samples from Nebraska.

[b] No evidence of midge infestation but in which infestation by earlier generations was possible.

[c] Infested by diapausing larvae.

Fremont County, Iowa. The average percentage of normal florets for all samples was 34.5%.

It was not possible to make a comparison between most of the data collected in 1965 and that collected in 1966 because the samples were collected in different areas of the state. However, the sample collected from Lancaster County in 1965 and Lancaster County sample No. 1 collected in 1966 came from the same field and the same sampling technique was used in both years. The percentage of florets infested with diapausing larvae was nearly three times higher in 1966. It would seem logical that the earlier generations should also be composed of greater numbers, thus causing more undeveloped florets in 1966. This, however, was not the case as the rate of undeveloped florets was considerably higher in 1965. Because factors other than midge infestation undoubtedly play an important part in bromegrass seed set, it is quite difficult to compare the damage caused by the midge from year to year. Nevertheless our data show that infestations can be as high as 50%. Agafonova (2) reported that in Russia infestation rates in certain years reaches 100%.

Nielsen and Burks (9) reported that a seed set of 65 to 70% was considered satisfactory for smooth bromegrass in Wisconsin. The percentage of normal florets in Nebraska in 1965 and 1966 was considerably less than this. While we cannot determine how many of the undeveloped florets resulted from midge infestation, it was concluded from the number of diapausing larvae found that the midge contributed significantly to the number of florets that failed to develop normal seed.

While taking sweep net samples for adult midges in a Lancaster County field in 1966, it was noted that a great many florets were shattering from the panicles and were being collected in the sweep net. An examination of the collected florets showed that the floret adjacent to the broken rachilla joint was usually infested by a diapausing midge larva. The rachilla joint immediately below the infested floret was weakened and the floret shattered easily from the spikelet.

This phenomenon was not observed in non-infested florets or in florets infested by the non-diapausing larvae of the midge. The specific cause of shattering is unknown. However, the effect of the seed shattering on the amount of seed harvested may be significant since all florets distal to the broken rachilla joint will be lost with the infested floret whether they are infested or not.

The shattering of the seed seemingly caused by the presence of the diapausing larvae appears to be another example of the close relationship that exists between *S. bromicola* and its host plant.

The shattering of the florets containing the diapausing larvae places them on the ground surface where the larvae may be more protected from adverse winter weather and where they are much more likely to receive the moisture that appears necessary for breaking diapause (see Table 2) in the spring.

Observations made late in the fall showed that many of the florets had shattered from unharvested panicles and very few diapausing larvae were found in the panicles. Since shattering begins before the normal harvest of bromegrass seed, it is probable that seed loss may be considerable.

The observed damage caused by the feeding of the midge larvae plus the suspected loss of seed due to the shattering of the florets combined to make *S. bromicola* a major pest of smooth bromegrass seed production in Nebraska in 1965 and especially in 1966.

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