EFFECTS OF VARIOUS TEMPERATURES ON THE SURVIVAL OF THE WHEAT CURL MITE, ACERIA TULIPAE (KEIFER)

by

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INTRODUCTION

Eriophyids have an extreme host specificity, some species parasitize only certain species of one plant genus, others infest several genera but rarely does one species have more than one host plant family as they have no way of surviving long periods without living host plants. They are all essentially parasites of perennial plants (18) but are on some annuals which become infested by mites from perennials. Thus,eriophyids sometimes have an annual favorable for their growth which overlaps with another annual. This, for example, is how Aceria tulipae (Keifer) has been able to survive Western United States wheat areas (31).

Studies of their relationship to temperature and other environmental factors become important in understanding the biology and ecology of Aceria tulipae (K.) vector of wheat streak mosaic virus (WSMV) in knowing how to control the mites and the disease it transmits. Previous workers (31, 32) have reported that destruction of volunteer wheat and timely seeding of the wheat crops was found effective in controlling WSMV, but in 1959, in Kansas the practice became difficult to achieve because of the prevailing conditions, where summers were relatively cool and moist, where fall was long, warm and moist that favors the continued growth of volunteer wheat, survival and astronomical multiplication of the vectors and dissemination of the mites (19, 26). In southern Alberta, in 1963, (2) the most severe outbreak of wheat streak mosaic was brought upon by a unique succession of unusual weather conditions, a prolonged period of exceptionally warm weather that favored the continued multiplication and dispersal of viruliferous mites throughout the winter fields.

There are now seven known viruses of plants transmitted by eriophyid mites (25, 31). The best known mite-vector of plant viruses is the wheat curl mite, Aceria tulipae (Keifer), the efficient vector of wheat streak mosaic virus (WSMV) (6, 8, 29, 32). The losses caused by this disease are very serious in the Great Plains in the United States and Canada. Other countries have been reported where WSMV may have been identified outside North America. They are Rumania, Jordan, and possibly a disease of wheat in the Krasrodar region in the USSR (31). It is now reported further east in the United States, as far as Ohio (37), Indiana, and Michigan where it has been detected both on wheat and corn. WSMV was probably observed as early as 1929 in Kansas and maybe before that in Nebraska (20). In 1949 alone, the disease caused damage of approximately 30 million dollars (14). And from time to time has caused serious losses in other states over the Great Plains including Nebraska (32), South Dakota (28), Colorado (1), Montana (21), Wyoming (35). It was also found and reported in western North Dakota (33), Washington (4), Idaho (12), and California (15). The southern boundary was northern Oklahoma (34) and the Texas panhandle (3). Since the demonstration of mite transmission of WSMV, A. tulipae has been found the vector of WSMV everywhere it has been tested in the United States. In addition, the same species of mite is the only known vector tested of a virus identified as WSMV in Rumania in 1960 and in Jordan in 1961 (31). It was also reported as vector of wheat spot mosaic in Canada (29). Only recently, A. tulipae was reported to secrete phytotóxic materials into corn, resulting in kernel red streak in Ohio (22).

Nothing was known about the means of overwintering nor the temperature tolerance of the developmental stages of the mite when it was first discovered as a vector of WSMV. Later, when this was confirmed as the efficient vector of WSMV, several workers were stimulated to study the biology and ecology of A. tulipae. The present study is a continuation of the work undertaken on the biology and ecology of the wheat curl mite by previous workers (10, 13) at Kansas State University, particularly the effects of various temperatures on the survival of the different developmental stages of this mite. Because of the scarcity of information concerning this phase of the problem, this study of the effects of sub-freezing, near-freezing and high temperatures was conducted. It may also reveal some information that may be valuable to help answer the following questions: (a) the unexplained cyclic pattern of population fluctuations often experienced by workers growing or observing A. tulipae in both greenhouse and field, (b) the rapid rise of mite population in some epiphytotic years during late August and September (27).

REVIEW OF LITERATURE

Description of the Mite

Members of the suborder Prostigmata, family Eriophyidae, are microscopic, soft and worm-like, and have only two pairs of functional legs, both of which are located at the anterior end of the body. The posterior pair is reduced to fine hairs or are missing. A short cephalothorax bears the legs, a pair or short jointed palpi and the rostrum. The abdomen often has concentric lines and bears a number of long spines and truncate telson from which two caudal spines arise. An anal sucker is located at the end of the abdomen and functions as an anchor, keeping the mites from being accidentally dislodged from plants. All known members are plant feeders, a few of them live on tender twigs, some live freely upon leaves, and others feed on fruits or buds (5, 11). Various species damage plants by the production of galls, blisters, bud swellings, rolled leaves, russeting, and erineum (a thick growth of felt hair).

The mite was first described as Eriophyes tulipae (Keifer), (16). Keifer (17) separated Aceria from Eriophyes because Aceria has a rear shield margin of the dorsal setiferous tubercles which direct the setae caudad. The name Aceria is composed of the first two letters of Acarus and the first part of Eriophyes. The original specimen was collected from tulip bulbs at Sacramento, California. It is believed that the first mite described came from Holland in tulip bulb shipments (16).

A. tulipae is 150 to 270 microns in length and can hardly be seen by the naked eye when occurring singly. The cephalothoracic shield has a short lobe projecting over the base of the chelicerae. This projection is visible only when the rostrum and chelicerae are pulled down from the front shield, which usually does not occur naturally (18). Abdominal segments do not have distinguishable tergites and are always microtuberculate. The cover flap of the genetalia may be smooth or furrowed (7). Reproduction is parthenogenic, the eggs are spherical, 30 to 50 microns in diameter and turn pearly white near hatching. They are laid in close association with the adults and are cemented lightly to the plant surface (30).

Life Cycle

A study of the life cycle of A. tulipae was reported by Staples and Allington (32). They found the salient details of the life cycle from egg to egg at 75°F. to 78°F. to be as follows:

Complete cycle from egg to egg . . . . 8 to 10 days

Eggs incubation period . . . . 3 days

First nymph . . . . $1\frac{1}{2}$ days

First molt . . . . 3/4 day

Second nymph . . . . $1\frac{1}{2}$ days

Second molt . . . . 3/4 day

Egg hatching to adult . . . . 4 to 5 days

Preoviposition period . . . . 1 to 2 days

Slykhuis (30) and Staples and Allington (32) were not able to determine exactly the number of eggs laid by a single female mite. The latter, however, estimated that about 12 eggs were produced by a single adult. Del Rosario and Sill (10), reported later that as many as 21 eggs and as few as 3 eggs were laid by individual females, and that laying occurred largely during early morning and late afternoon. The majority of the eggs were laid on the first laying day.

Effects of Temperature and Humidity on Mite Survival

Information regarding the effects of temperature and humidity on the wheat curl mite is meager. Slykhuis (30) stated that eggs hatched readily in near 100 per cent humidity at 77°F., but mites perished at low humidities (50 per cent and below at 77°F.), and further observed that nymphs and adults were killed between -15° and -18°F. However, eggs were still viable to about -23°F. for 2.5 minutes and hatched when incubated at room temperature. Del Rosario and Sill (10) reported that mites grew satisfactorily in the greenhouse during summer months when temperatures occasionally reached 120°F., that adults survived at least several months on leaves at usual refrigerator (35° to 39°F.) temperatures. Del Rosario (8) reported also that hatching of eggs was best at a temperature of 75±5°F. and very low at near freezing temperature (35±5°F.) and that some eggs kept at near freezing temperature for 1 to 2 weeks were able to hatch after being returned to room temperature. Del Rosario stated that egg hatching was much delayed at 32±5°F.; at 48±5°F. it took 3 days; and at 75±5°F. hatching occurred rapidly in only 1 to 2 days. Adults exposed at 32±5°F. did not reproduce normally but survived, an indication of hibernation. At 48±5°F., they increased in numbers slowly but did not become as numerous as at 75±5°F. Gibson (13) observed in the field that mites persisted on volunteer wheat plants throughout the winter months as eggs and adults. Gibson's studies also showed that when air temperature in Kansas wheat fields during the summer of 1954 and 1955 exceeded 100°F. mites reproduced poorly and populations dwindled even on wheat, which is an excellent host. Staples and Allington (32) also reported a sudden, unexplained decrease in mite populations on volunteer wheat during the summer.

MATERIALS AND METHODS

Mites and Eggs Used

The mites for this study were collected from wheat fields in various sections of Kansas during the summer of 1964. They were maintained in the greenhouse on potted wheat plants covered with plastic cages. No attempt was made to control the age of the eggs used in the various egg experiments.

Caging Technique

The cages were made of plastic sheets which were rolled around an ordinary half gallon Ball jar as a pattern. The edges were pasted with acetone to hold the cage as formed. Each cage was 3½ inches in diameter and 9 inches high. The top of each was covered with nylon taffeta cloth pasted on the top opening with acetone. This cloth cover provides free circulation of air inside the cage for the mites and the plants. Another cage used was an ordinary lantern globe or lamp chimney also used as a rearing cage by Del Rosario and Sill (9). The bottom of the globe was pushed into the soil around the growing plants and the top was covered with nylon taffeta.

Maintenance of Mite Cultures in the Greenhouse

The technique described by Del Rosario and Sill (9) of shifting mites from old cultures to fresh, growing young plants was continued as Slykhuis (30) reported that mites tended to decrease in population as plants grew to maturity.

Petri dishes were used in testing the hatchability of eggs at both low and high temperatures. Prior to using, they were thoroughly cleaned with detergent and rinsed several times under hot running water.

Techniques Employed in Testing the Effects of Continuous Sub-freezing Temperatures on the Hatchability of Eggs of the Wheat Curl Mite

Eggs for studying the effects of sub-freezing, near-freezing and high temperatures were picked up singly under the binocular microscope from the cultures maintained in the greenhouse using the methods described by Del Rosario and Sill (9). Fifty eggs were transferred to excised wheat leaves in Petri dishes layered at the bottom with moistened filter paper. The Petri dishes then were placed inside a freezer at the desired temperatures. Cut leaves were taken out as desired after subjecting them to different temperatures (23°, 14°, and -4°F.) for a period of 1, 2, 4, 8, 16, and 32 days). The removed cut leaf was transferred to a new Petri dish also layered at the bottom with moistened filter paper. Immediately after removal from the freezer the eggs were examined to see whether they had hatched while inside the freezer. After examination they were kept at greenhouse temperature (75±5°F.) where daily observations for hatching of eggs were recorded for a period of 32 days. Eggs were transferred to newly cut leaves every 4 to 6 days to provide the eggs and nymphs that would hatch with fresh leaves. Two replicates were carried out for this experiment in each trial.

Techniques Employed in Testing the Effects of Alternate Daily Shifting of Eggs from Sub-freezing Temperatures to Greenhouse Temperature

To study the effects of alternate daily shifting of eggs from each of the sub-freezing temperatures (23°, 14°, and -4°F.) to greenhouse temperature, eggs were collected in the same manner as described for the previous test, but were held for 16 hours at freezing temperatures and then brought to greenhouse temperature for 8 hours each day. Immediately following removal from the freezer the Petri dishes were uncovered and examined carefully to see whether the eggs hatched inside the freezer. Then they were kept at the greenhouse temperature after which hatching was recorded prior to returning the eggs to the freezer. Eggs kept at the greenhouse temperature were used as a control for the experiments.

Techniques Employed in Testing Alternate Daily Shifting of Eggs from Freezing and Near-Freezing, to Room Temperature

To determine the effects of alternate daily shifting from near-freezing and freezing temperatures (36° and 32°F., respectively) to room temperature, the eggs were handled as previously discussed. Eggs that hatched inside the freezer (32°F.), or near-freezing (36° F.) were recorded separately after each removal from the temperature under test.

Techniques Employed Concerning the Effects of High Temperatures on the Hatchability of Eggs

To determine the effects of high temperatures on eggs, (115±5°, 95±5°, 85±5°, and 75±5°F.), control chambers were used. The greenhouse temperature of 75±5°F. was used as the control. Eggs for this experiment were collected as described previously. Daily observations of the eggs which hatched inside the Petri dishes were recorded. Eggs were transferred to newly cut leaves every 4 days or as often as necessary when the leaves became water-soaked and yellow. The filter paper was always kept moist at each temperature to provide high humidity for the eggs.

Relative Humidity

In the experiments using the different temperatures the relative humidity was assumed to be nearly 100 per cent inside the Petri dishes because of the frequently moistened filter paper lining the bottom of each Petri dish.

Techniques Employed in Testing the Effects of High Temperatures on Nymph Maturation Reproduction and Survival

The nymphs were picked up singly from the cultures maintained in the greenhouse and 5 to 10 nymphs were placed at the base of the youngest leaf on each of the potted wheat plants bearing 3 to 4 leaves. These seedlings were divided equally into four groups. Each group was maintained at either 75±5°, 85±5°, or 115±5°F. The seedlings were covered with plastic cages and the pots were held in an earthen trough which was used for watering. Observations regarding the survival and population development of nymphs were recorded after 11 days.

Techniques Employed in Testing the Effects of High Temperatures on Adult Survival and Reproduction

In another series of experiments adults were used to study the effects of high temperatures (75±5°, 85±5°, 95±5°, and 115±5°F.) on the survival and reproduction of the mites. The methods and procedures applied were exactly the same as for the nymphs.

In both the nymph and adult tests, it was necessary to pull the whole plant and tear off the leaves one by one for examination under the microscope. The leaf sheaths below the ligule were carefully examined since the mites tend to migrate downward into the heart of the plant as temperature go higher.

EXPERIMENTAL RESULTS

Effects of Continuous Sub-freezing Temperatures on the Hatchability of Eggs of the Wheat Curl Mite

In the experiments concerning continuous sub-freezing temperatures on eggs, the temperatures used were 23°, 14°, and -4°F. for periods of 1, 2, 4, 8, 16, and 32 days. After removal from these temperatures to 75±5°F., observations were made for 32 days on the hatchability of the eggs. The results obtained are presented in Table 1 (Appendix).

The data clearly demonstrate that by decreasing the sub-freezing temperatures from 23° to -4°F., the per cent hatch decreases and that when eggs were held constantly for 16 to 32 days at sub-freezing temperatures, they lost their viability, i.e., they did not hatch after removal from the freezer. It is also evident that a large percentage of the eggs at 23°F. remained viable for 8 days and thereafter the per cent survival dropped rapidly. All eggs, after removal from the freezer, were kept under observation at greenhouse temperature (75±5°F.) for a period of 32 days.

The results reported here are in basic agreement with the findings of Slykhuis (30) concerning the effects of sub-freezing temperatures on the survival of A. tulipae.

Effects of Alternate Daily Shifting of Eggs from Sub-freezing Temperatures for 16 Hours to Greenhouse Temperature for 8 Hours

In these experiments the eggs were subjected to the following sub-freezing temperatures: 23°, 14°, and -4°F. The greenhouse temperature was 75±5°F., which was also used for the controls. The number of eggs used for the test at 23°F. was 600, for 14°F. 135, for -4°F 250 and for the control at greenhouse temperature 200. As shown in Table 2, when the eggs were shifted from 23°F. to greenhouse temperature and back, the results showed a prolonged period of hatchability, extension of incubation period and survival of some few even up to 10 days. The percentage eggs hatched after the first exposure at this temperature has a marked increase and continued to the 4th day. Then the egg-hatch thereafter decreased and completely suspended after 10-day period of back and forth shifting under those temperatures. The same trend of increase at 14°F. after the first egg hatched was recorded on the 3rd and 4th day of observation and thereafter decreased and stopped after 7 days. The eggs tested with -4°F. did not hatch during the first day of observation, but showed many eggs hatched the 2nd day and abruptly decreased on the 3rd day and was stopped after the 4th day. Apparently the experiments revealed that sub-freezing temperatures did not seem to have an adverse effect in the hatchability of the eggs in the second day of observations in all temperatures tested. Lowering the sub-freezing temperatures, however, to -4°F. even while maintaining the greenhouse temperature has killed many eggs after the 4th day and showed the lowest percentage hatched at the end of the experiments. The majority of the eggs also, if viable, hatched in 1 to 7 days. The eggs held constantly under the greenhouse condition showed a total per cent hatch of 48%.

All the remaining unhatched eggs after 32 days were kept at greenhouse temperatures for a period of 2 weeks. No hatching was recorded during this period, hence they were all assumed to be dead. It should be mentioned that the hatching always occurred when the eggs were brought back to the greenhouse and kept for a period of 8 hours and not during the period when they were inside the freezer.

<u>Effects of Alternate Daily Shifting of Eggs to Freezing or Near-freezing Temperatures for 16 Hours to Room Temperature for 8 Hours</u>

To determine the effects of alternate daily shifting of eggs of the wheat curl mite to freezing or near-freezing temperatures, 130 eggs were subjected to 36°F. for 16 hours and to room temperature, 68±3°F. for 8 hours. The results obtained are presented in Table 3 (Appendix). The data in Table 3 indicate that at 36°F. there was a 12 per cent hatch during the period when the eggs were held at near-freezing in the refrigerator for 16 hours. The hatching occurred on the 4th and 6th day inside the refrigerator and never thereafter. Most eggs hatched at room temperature. There was an 84 per cent total hatch at room temperature while the eggs were held at 68±3°F. for 8 hours each day. The majority of the eggs hatched at room temperature from the 2nd to the 7th day of observation. No egg hatched after 8 days at either 36°F. or 68±3°F. The observations were carried on for a period of 32 days and the 4 per cent which did not hatch at either temperature were assumed to be dead.

In another series of experiments, 115 eggs were subjected to 68±3°F. (room temperature) and 32°F. (freezer temperature) following the same alternate daily shifting procedure as described in earlier experiments. The results are given in Table 4 (Appendix). At room temperature a total of 83 per cent hatch was recorded while only 7 per cent of the eggs hatched at 32°F. The majority of the eggs hatched in from 2 to 7 days at room temperature while at the freezer temperature, hatching was very low and occurred between the 4th and 6th day. In both the experiments (Table 3 and 4) the remaining unhatched eggs were removed from the freezer and were kept at room temperature for a week. Daily observations up to 7 days yielded no hatched eggs, thus all eggs were discarded and assumed dead.

<u>The Hatchability of Eggs Held Continuously at Freezing or Near-freezing Temperatures</u>

In this study, 50 eggs were used for each of the two temperatures tested (32° and 36°F.). The eggs were held continuously for 32 days inside the freezer and daily observations for hatching were recorded. The results are given in Table 5 (Appendix). The data in Table 5 show a 12 per cent and 4 per cent hatch at 36° and 32°F. respectively, and a total of 8 per cent. In both temperatures under trial the hatch was very low. It is interesting to note that at both temperatures hatching was recorded even at the 17th day and at the 20th day at 32°F. After 32 days the unhatched eggs were taken out of the freezer and kept at room temperature to check whether the eggs were still viable. No hatching was observed during one week and all eggs were assumed to be dead.

<u>Effects of Continuous High Temperatures on the Hatchability of the Eggs</u>

To determine the effects of high temperatures on the hatchability of eggs, the following temperatures were used: 115±5°, 95±5°, 85±5°, 75±5°F., and the control was held at greenhouse conditions at 75±5°F. The number of eggs used in each treatment was 400, 325, 250, 100, and 100 respectively, according to the availability of eggs. The eggs were held at the temperatures listed above for 32 days and observations were recorded daily. The results obtained are tabulated in Table 6 (Appendix).

The results in Table 6 show only a 54.7 per cent hatch at 115±5°F. This gradually increased to 58.4 per cent, 66.6 per cent and 77 per cent at 95±5°, 85±5°, and 75±5°F., respectively. Under the control conditions at 75±5°F. the hatching was highest, or 84 per cent. Hatching occurred mostly in 1 to 3 days at these temperatures, which is in agreement with the findings of Del Rosario (8). Furthermore, it was revealed that there was no hatching after 9, 5, 4, and 7 days at 115±5°, 95±5°, 85±5°, and 75±5°F., respectively. The eggs which did not hatch during the 32 days were recorded as dead. The ideal temperature for hatching based upon all work done so far would be between 70° and 80°F.

Effects of High Temperatures on the Maturation and Reproduction of Nymphs

To study the influence of high temperatures on the maturation and reproduction of the nymphs, the following temperatures were used: 75±5°, 85±5°, 95±5°, and 115±5°F. The young plants were infested with 5 to 10 nymphs each and the populations were recorded after 11 days. The results are presented in Table 7 (Appendix). The data show that only a few nymphs tested at 115±5°F. were able to mature or reproduce. The numbers for eggs, nymphs and adults were very low as compared to the other temperatures tested. A high population of eggs after 11 days was found at the other three temperatures (75±5°, 85±5°, and 95±5°F.). Nymph populations developed somewhat more favorably at the temperature 95±5°F. but almost equally well at 75±5°, and 85±5°F. Adults produced were nearly the same at 85±5°F and 95±5°F. temperatures and slightly higher at 75±5°F. The deleterious effects of the 115±5°F. temperature for eggs, nymphs, and adults is very clear.

Effects of Continuous High Temperatures on the Reproduction and Development of Adults

In another series of experiments the influence of continuous high temperatures were tested using the same temperatures as with the nymphs. In each of the 12 trials, 12 plants were infested with 5 to 10 adults for each temperature.

In this series of experiments adult mites appeared to develop more favorably again at 95±5°F. than at 75±5°F., the same as for the nymphs. At 115±5°F. population development was again very poor, just as it was when the nymphs were used. The observation strongly suggests that the three lower temperatures (75±5°, 85±5°, and 95±5°F.) tested would support the reproduction and population development of adult mites as well but not 115±5°F.

DISCUSSION AND SUMMARY

Experiments were conducted to determine the effects of sub-freezing, freezing, near-freezing and high temperatures on the hatchability of the eggs of the wheat curl mite, Aceria tulipae Keifer.

When the eggs were exposed continuously to various sub-freezing temperatures, (23°, 14°, and -4°F.) the per cent hatch decreased as the temperature decreased. When eggs were held constantly from 16 to 32 days at these temperatures they lost their viability. Many eggs at 23°F., however, remained viable for 8 days. These results indicate that eggs can tolerate sub-freezing temperatures for a time and still be able to hatch when incubated at above freezing temperatures. There was a sudden rise of the per cent hatch observed on the second day of exposure at 23°F. which could possibly be due to the undetermined ages of eggs in this experiment. Most eggs in this group may have already gone through the development of their embryo and may have been about to hatch while they were being picked up for use and when subjected to sub-freezing conditions, a hibernating stage was simulated. Upon removal then to greenhouse temperatures (75±5°F.) those eggs suddenly emerged. The remainder, however, that did not hatch were presumed to have been killed by the temperature used. The above results also would show how temperature in the field would affect the survival of the eggs during cold weather. The cold weather could persist up to 8 days, both day and night, and then a sudden rise of temperature above freezing after this period still would enable most of the eggs to hatch. It also implies that overwintering eggs at very low temperatures would not be viable after a week of continuous sub-freezing temperatures.

The alternate daily shifting of eggs of the wheat curl mite from sub-freezing temperatures (23°, 14°, and -4°F.) for 16 hours to greenhouse temperature (75±5°F.) for 8 hours during a period of 32 days revealed that the overall total percentage egg-hatch decreased when the temperatures were lowered to -4°F. The hatching recorded occurred only at the greenhouse temperature during the 8 hours each day and not during the period when the eggs were held at sub-freezing temperatures. The highest total percentage egg-hatch was recorded at 23°F., and about 58 per cent. The greenhouse temperature (75±5°F.) used as a control showed a total percentage egg-hatch of 47.5. The difference may have been due to the lower relative humidity under the greenhouse conditions or temperature. Slykhuis (30) pointed out the importance of relative humidity in his experiments when eggs hatched readily at 77°F. when the relative humidity was 100 per cent, but hatching was lowered under the same temperature when the relative humidity was about 75 per cent and hatchability was completely arrested when the relative humidity was lowered to 50 per cent or lower. In the present experiment the results also showed that sub-freezing temperatures and alternate shifting of the eggs from low to high temperatures and vice versa delayed the normal hatching period of eggs as compared to the more favorable condition (77°F.) when eggs hatched in 3 days as reported by other workers. The current results also indicated the ability of the eggs to survive extended periods of cold temperatures and then hatch when the temperatures were raised. In this experiment then, a 16 hours sub-freezing temperature in the field could prevail at maybe, night time during cold season and with a sharp rise in temperature at daytime would still favor the mite egg to hatch. At about 23°F. especially, more than 50 per cent of the mite eggs in the field may still hatch under such cold and warm alternation of the temperatures. However, more than a week of such sub-freezing temperatures alternating with warm days would be deleterious to egg survival.

Experiments on the effects of daily shifting of eggs of the mite to near-freezing (36°F.) or freezing temperature (32°F) for 16 hours and to room temperature (68±3°F.) for 8 hours, indicated that in both freezing and near-freezing trials the hatching was very low when compared to the room temperature egg hatched. Few eggs are able to hatch at either temperature. Possibly when the eggs were removed to room temperature, the development of the embryo in some of the eggs reached a point where the eggs were about to hatch and just as they were returned to the freezer, they emerged. This is not known to be the case, however, as the hatch was never recorded until the end of the 16-hour period in the freezer.

When the eggs were held continuously at 36°and 32°F. the results show that at least some of the eggs can hatch at freezing or near-freezing temperatures, which further indicates much individual egg variation in ability to resist cold temperatures. The results obtained here substantiated the findings of Del Rosario (8) who reported that hatching was lowest at near-freezing temperatures and was much delayed.

In the series of experiments with high temperatures the hatchability of eggs was 23 per cent lower at 115±5°F. than at 75±5°F. This demonstrates that very high temperatures as well as very low temperatures reduced the viability and hatchability of the eggs. Eggs maintained at the greenhouse temperature (75±5°F.). as a control showed a total per cent hatch of 7 over the 75±5°F. controlled chamber, which appeared better than the rest of the temperature tested. The greenhouse temperature though, may have fluctuated either above or below during the time the experiment was under observation, which would probably account for the difference in hatch under the controlled chamber of 75±5°F. Here, a sudden rise of egg hatch was observed on the second day at 115±5°, 95±5°, and 85±5°F. The different ages of the eggs used in this experiment could possibly be the explanation. That is, most of the eggs must have gone through their embryonic development and were about to hatch when subjected to these temperatures. The unusually high temperature may have stimulated the eggs to hatch quickly. The rest of the eggs that did not hatch on the second day may have been in their immature germinal cell and had to wait or undergo the developmental process. This would account for the prolonged incubation period.

The overall results indicate a hardiness of the eggs under unfavorable conditions. Of course, since no actual measure of the relative humidity was made, this may be another important factor. The laboratory materials like Petri dishes and the handling of these delicate arthropods while picking them up singly with a pointed hair attached to a wooden handle may have destroyed mites also.

In the study on the effects of high temperatures on the maturation and reproduction of nymphs and adults, the temperatures used were: $75 \pm 5^{\circ}$, $85 \pm 5^{\circ}$, $95 \pm 5^{\circ}$, and $115 \pm 5^{\circ}$F. The results concerning the population of eggs, nymphs, and adults produced after 11 days showed that both nymphs and adults did not reproduce well at $115 \pm 5^{\circ}$F. when compared to the other temperatures tested. The highest population of eggs, nymphs, and adults was found to be at $95 \pm 5^{\circ}$F. The nymphs and adults at $85 \pm 5^{\circ}$ and $75 \pm 5^{\circ}$F. reproduced quite well and more or less equally.

In the adult mite test the same result was obtained as with the nymphs with regard to the effect of $115 \pm 5^{\circ}$F. temperatures; and $95 \pm 5^{\circ}$F. again favored the adult reproduction and development. The results obtained in both the nymph and adult experiments indicate that at very high temperature, $115 \pm 5^{\circ}$F., the development and the reproduction of the mite was greatly retarded. It, therefore, supports the observation of Gibson (13) who stated that when summer temperatures exceeded 100°F. in the field, mite population was very low. Also it may explain the observation of Staples and Allington (32) of low mite population in volunteer wheat during a hot summer.

The importance of the whole study undertaken could very well be applied to support the different reports from various workers of WSMV and the vector. In Nebraska, Weighing (36) reported that the mites do not "freeze out" during Nebraska winters. Orlob (23) in South Dakota reported that WSMV damage in 1964 was attributed by plant pathologist mainly, to unusually warm temperatures through the preceding October. With the arrival of cool temperatures in the fall, mites became less active and reproduced much less rapidly, thus virus spread was reduced accordingly. Sill (26) also reported that when the summer was relatively cool and moist, volunteer wheat grew well and in great quantities in Western Kansas, which supported the mite populations in the field. This type of condition is favorable for the mites to reproduce most rapidly, and by the end of summer of 1958, it was observed that mites were in astronomical numbers in Kansas.

Previous experiments also showed that WSMV persists for some time in the vector, depending upon the temperature (8, 30). Recently it was reported by Orlob (24) that A. tulipae kept at room temperature remained viruliferous for at least 7 days; at 37.4°F. it remained infective for 61 days. In the present study, when eggs were held continuously at 36°F. for 32 days, the eggs showed variability in resisting cold temperatures, but some were able to hatch and thus could remain infective for as long a period as reported by Orlob, if those eggs hatched on a diseased wheat plant. Because nearly all individuals are females, (32) each of these individuals could produce an average of 12 eggs, and with the life cycle of 8 to 10 days from egg to egg, a single mite would be expected to produce approximately 180 quadrillion descendants in 50 days under favorable conditions. Hence, an epiphytotic of WSMV in the field, if both vector and virus are present, could be expected. The overall results of the whole work presented has shown a variable tolerance of this species of mite to adverse conditions. The data observed would substantiate mite survival in the field, both in the cold season and during hot summer.

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APPENDIX

Table 1. Effects of continuous sub-freezing temperatures on the per cent hatchability of the eggs of the wheat curl mite.

Temperature used °F.	No. of eggs used	Days held at sub-freezing temperatures and number of egges hatched a/						Total eggs hatched	Percent eggs hatched
		1	2	4	8	16	32		
23°	600 b/	30	61	65	45	0	0	201	34
14°	600	15	12	12	4	0	0	43	7
-4°	600	6	12	1	1	0	0	20	3

a/ Number of eggs hatched during a 32-day period after removal from freezer on the day shown.

b/ Two trials including 600 eggs were conducted at each temperature. One hundred eggs were removed to room temperature on each of the days listed above.

Table 2. The effects upon hatchability of alternate daily shifting of eggs of the wheat curl mite from sub-freezing temperatures (16 hours) to greenhouse temperature (8 hours).

Temperatures used (°F.)	No. of eggs used	Percentage egg hatch each day												Total percent hatch
		1	2	3	4	5	6	7	8	9	10	11	32	
23	600	3.1	13.0	10.3	15.7	9.5	3.5	1.5	0.5	0.2	0.2	0	0	57.5
14	135	5.9	7.3	5.1	2.9	0.7	0.7	0.7	0	0	0	0	0	23.3
-4	250	0	14.4	5.6	0.4	0	0	0	0	0	0	0	0	20.4
75±5 a/	200	4.0	21.0	8.0	0.9	1.5	0	0	0	0	0	0	0	47.5

a/ Greenhouse temperature for controls and for 8-hour daily greenhouse exposure.

Table 3. Effects upon hatchability of alternate daily shifting of eggs of the wheat curl mite from near-freezing (16 hours) to room temperature (8 hours).

Temperature used °F	No. of eggs used	Number of eggs hatched per observation on each of following days											Total No. eggs hatch	Per cent hatch
		1	2	3	4	5	6	7	8	9	10	32		
Room Temp. (68±3°F.)	130	0 a/	10	8	17	17	27	30	0	0	0	0	109	84
Refrigerator (36°F)		0 b/	0	0	9	0	7	0	0	0	0	0	16	12
													Total - - - - - -	96

a/ Eggs hatched during room temperature observation period.
b/ Eggs hatched during refrigerator temperature observation period.

Table 4. Effect upon hatchability of alternate daily shifting of eggs of wheat curl mite to freezing (16 hours) and to room temperature (8 hours).

Temperature used °F.	No. of eggs used	Number of eggs hatched per observation on each of following days											Total No. eggs	Per cent hatch
		1	2	3	4	5	6	7	8	9	11	32		
Room Temp. (68±3°F.)	115	0 a/	4	17	15	12	21	3	1	0	0	0	73	63
Freezer (32°F.)		0 b/	0	0	2	1	5	0	0	0	0	0	8	7
													Total - - - - - -	70

a/ Eggs hatched during room temperature observation period.
b/ Eggs hatched during freezer temperature observation period.

Table 5. Effects of continuous temperatures of 32°F. and 36°F. on the hatchability of the eggs of the wheat curl mite.

Temperature used °F	No. of eggs used	Number of eggs hatched each day at temperature shown													Total No. eggs hatched	Per cent hatch
		1	2	3	4	5	6	10	11	16	17	20	21	32		
36°	50	0	1	0	1	2	0	1	0	1	0	0	0	0	6	12
32°	50	0	0	0	0	0	0	0	0	1	0	1	0	0	2	4

Table 6. The effects of continuous high temperatures on the hatchability of wheat curl mite eggs.

Temperature used (^{o}F.)	No. of eggs used	1	2	3	4	5	6	7	8	9	14	32	Total per cent hatch
115±5	400	5.5	19.2	18.7	4.0	4.5	1.5	0.5	0.5	0.3	0	0	54.7
95±5	325	17.2	32.6	6.4	0.9	1.2	0	0	0	0	0	0	58.3
85±5	250	13.2	46.6	6.4	0.4	0	0	0	0	0	0	0	66.6
75±5	100	34.0	21.0	7.0	10.0	2.0	2.0	1.0	0	0	0	0	77.0
75±5^{o}F. a/	100	41.0	28.0	7.0	6.0	2.0	0	0	0	0	0	0	84.0

a/ Greenhouse temperature controls . (Temperatures were occasionally higher)

Table 7. Effects of continuous high temperatures on the maturation of wheat curl mite nymphs.

Expt. No.	No. of nymphs used	Number of eggs, nymphs, and adults observed after 11 days at the following temperatures: 75±5°F.			85±5°F.			95±5°F.			115±5°F.		
		Eggs	Nymphs	Adults	Eggs	Nymphs	Adults	Eggs	Nymphs	Adults	Eggs	Nymphs	Adults
1	5 to 10	10 a/	4	10+	10+	3	2	10+	7	7	0	0	1
2	do	10+	10+	10+	10+	1	4	3	4	1	0	0	0
3	do	0	0	4	4	2	10+	6	7	7	0	0	0
4	do	10+	10+	10+	7	4	1	3	1	0	0	1	0
5	do	4	1	2	3	2	2	3	1	1	0	0	2
6	do	10+	1	3	10+	8	7	2	1	1	0	1	1
7	do	10+	0	0	10+	10+	10+	10+	10+	10+	1	0	0
8	do	1	1	10+	0	1	3	10+	8	10+	0	0	0
9	do	9	4	3	10+	1	1	10+	3	4	0	0	1
10	do	2	1	3	2	0	0	7	2	1	1	0	0
11	do	1	0	1	1	1	0	10+	0	2	0	0	0
12	do	0	0	0	0	0	0	3	0	4	0	0	0
TOTALS		67	32	56	67	33	40	76	44	48	2	2	5

a/ More than 10 individuals not counted

Table 8. Effects of continuous high temperatures on the reproduction and population of wheat curl mite adults.

Expt. No.	No. of nymphs used	Number of eggs, nymphs, and adults observed after 11 days at the following temperatures 75±5°F.			85±5°F.			95±5°F.			115±5°F.		
		Eggs	Nymphs	Adults	Eggs	Nymphs	Adults	Eggs	Nymphs	Adults	Eggs	Nymphs	Adults
1	5 to 10	0	0	2	6	9	10+	2	1	0	1	1	2
2	do	4	0	1	6	9	10+	2	1	0	1	1	1
3	do	4	1	4	1	2	1	2	0	0	3	0	3
4	do	1	1	0	3	2	4	1	2	0	1	0	0
5	do	10+[a]	0	0	6	0	2	10+	5	10+	1	0	0
6	do	3	3	5	10+	0	10+	1	1	1	3	0	0
7	do	0	2	3	3	0	4	5	4	6	3	1	0
8	do	2	1	4	5	2	9	10+	10+	10+	1	0	0
9	do	10+	10+	2	4	1	10+	10+	10+	10+	1	0	0
10	do	10+	2	3	0	1	1	5	1	2	0	0	0
11	do	10+	3	2	10+	0	1	10+	4	10+	0	0	0
12	do	0	1	1	2	0	2	10+	10+	10+	1	0	0
TOTALS		54	19	27	53	26	63	68	49	59	16	3	6

a/ More than 10 individuals not counted.

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EFFECTS OF VARIOUS TEMPERATURES ON THE SURVIVAL OF THE WHEAT CURL MITE, ACERIA TULIPAE (KEIFER)

by

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Studies on the effects of various sub-freezing, near-freezing and high temperatures on the survival of different developmental stages of Aceria tulipae (Keifer) were conducted. This study is a continuation of the work undertaken on the biology and ecology of the wheat curl mite by previous workers. The results obtained with sub-freezing temperatures (23°, 14°, and -4°F.) showed that eggs held constantly for 1, 2, 4, 8, 16, and 32 days can tolerate sub-freezing temperatures for 8 days and still be able to hatch when incubated at greenhouse temperature (75±5°F.). However, the eggs held for 16 and 32 days at these temperatures all lost their viability.

Experiments on the effects of daily shifting from sub-freezing temperatures for 16 hours to greenhouse temperature for 8 hours over a period of 32 days showed a 24 per cent hatch at 14°F. and 20 per cent at -4°F. At 23°F. the hatch was 58 per cent, and the incubation period was extended to 10 days. This indicates that sub-freezing temperatures delay the normal hatching period of eggs as compared to natural favorable conditions of 77°F. in which eggs hatch in about 3 days. The data also show that at least some of the eggs have the ability to survive sub-freezing temperatures for considerable periods and then hatch when the temperature rises above freezing.

When eggs were shifted daily to near-freezing (36°F.) or freezing temperatures (32°F.) for 16 hours and then to room temperature (68±3°F.) for 8 hours, the results showed that some eggs were able to hatch while at the cold temperatures, but the hatch was very low compared to that recorded when the eggs were kept at room temperature. At room temperature an 83 and 84 per cent hatch was recorded, while at refrigerator temperatures of 36° and 32°F., the per cent hatch was 12 and 7 per cent, respectively.

Eggs held continuously at near-freezing and freezing temperatures for a period of 32 days yielded a very low per cent hatch. Only 12 per cent of eggs kept at 36°F. hatched and only 4 per cent at 32°F.

Definitely the results show that some of the eggs are not killed by low temperatures. There was also a great variation in the hatching period of those few that survived, but it was consistently much delayed.

At high temperatures (75±5°, 85±5°, 95±5°, and 115±5°F.) eggs kept constantly at 115±5°F. gave the lowest per cent hatch. The temperature that appeared to be ideal for egg hatching was 75±5°F. Increasing the temperature from 75±5°F. to 115±5°F. decreased hatch by 22 per cent. Egg hatching occurred mostly in 1 to 3 days.

The effects of these constant high temperatures on the survival, maturation and reproduction of nymphs and adults showed that 115±5°F. is least favorable. The population of eggs, nymphs, and adults produced during 11 days were compared with the population produced at the other temperatures tested. The ideal temperature found for the maturation and reproduction of the highest population of eggs, nymphs, and adults was 95±5°F. although 75±5° and 85±5°F. were quite satisfactory temperatures.