THE MASS CULTURE AND FIELD RELEASE OF ANAPHES FLAVIPES (FOERSTER) (HYMENOPTERA: MYMARIDAE), AN EGG PARASITE OF THE CEREAL LEAF BEETLE, OULEMA MELANOPUS (L.) (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

THE MASS CULTURE AND FIELD RELEASE OF ANAPHES FLAVIPES (FOERSTER) (HYMENOPTERA: MYMARIDAE), AN EGG PARASITE OF THE CEREAL LEAF BEETLE, OULEMA MELANOPUS (L.) (COLEOPTERA: CHRYSOMELIDAE)

by Larry Carson Barton

Experiments have been conducted to improve the technique currently in use for mass culturing Anaphes flavipes (Foerster) (Hymenoptera: Mymaridae), an egg parasite of the cereal leaf beetle, Oulema melanopus (L.) (Coleoptera: Chrysomelidae). There are basically two techniques for rearing Anaphes: removing cereal leaf beetle eggs from plants upon which they are laid or leaving the eggs on the plants. Tests have shown that there is no difference between the two techniques in terms of the number of parasitized cereal leaf beetle eggs to develop per female parasite used. The removal of cereal leaf beetle eggs appears to be better than the non-removal of the eggs because (1) less space is required during rearing and during storage of both parasitized and unparasitized eggs, (2) eggs are easily counted, (3) it is easy to ascertain the number of parasites needed to parasitize the eggs, and (4) field release of parasitized material is easily accomplished.

Attempts were made to improve the techniques used in the storage of both unparasitized and parasitized cereal leaf beetle eggs. Storage techniques tested included (1) the use of modified gas atmospheres at both 14° and 40° F, (2) a glycerol egg-yolk-citrate extender to allow storage in liquid nitrogen, (3) the use of fungicides on cereal leaf beetle eggs stored at 40° F and (4) storage of eggs at 40° F while inverted over water.

During the spring and early summers of 1966 and 1967 field releases of Anaphes were made in various areas in southern Michigan. Although no overwintering adults were found in the spring of 1967, the releases in both 1966 and 1967 did indicate that Anaphes would successfully develop in the field and that possibly three generations could be obtained in one season.

The 1967 releases indicated that Anaphes will disperse at least 430 feet from the release point in one season. The releases showed that Anaphes can disperse through alternating 80 foot-wide strips of wheat, oats, and corn with voluntary oats.

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

Table | Page

LIST OF FIGURES

INTRODUCTION

The cereal leaf beetle, Oulema melanopus (L.), is native to the Old World and is found throughout most of Europe including England, Wales, and part of Siberia (Cooperative Economic Insect Report, 1958). In addition, it is found in Morocco, Tunisia, Iran, and Turkey (U.S.D.A. Program Aid 550, 1964).

The cereal leaf bettle (hereafter referred to as "CLB") was first discovered in the United States in southwestern Michigan. Although farmers were spraying for the CLB as early as 1959, it actually was not identified until 1962 from Berrien County, Michigan (Castro, 1964). Since then, the CLB has spread throughout the Lower Peninsula of Michigan and into Indiana, Ohio, and parts of Pennsylvania and Illinois (Cooperative Economic Insect Report, 1967). In addition the CLB has been reported in parts of Canada (Yun, 1967).

The cereal leaf beetle, a serious pest because of the preference for cereals by both the larvae and adults, also feeds on many other grasses (U.S.D.A. Program Aid 550, 1964). Early in the spring, as the overwintering adult CLBs become active, they concentrate their feeding on winter cereals but move to the more succulent spring grains such as oats when they develop.

Damage to winter grains is less severe because the plants are larger than the spring grain at the time of attack, and because of the movement of CLB adults to the spring grain. Damage to cereal crops in Russia has amounted to as much as 25 to 50% (Cooperative Economic Insect Report, 1958); the same source indicated that serious damage to cereal crops was recorded as early as 1891 in Hungary.

Because of the CLB's potential to damage cereal crops, a great deal of work has been expended in its control including the use of chemicals, plant resistance, and parasites. The work described herein has been conducted to develop and improve those techniques used for the mass culturing of Anaphes flavipes (Foerster) an egg parasite of the CLB. The work has included testing different containers, the storage of unparasitized eggs in modified gas atmospheres and under deep freeze conditions, storage of parasitized CLB eggs, and the use of fungicides on stored eggs. Tests were also conducted to discover whether Anaphes develops in eggs treated with up to 5000 roentgens X-ray. In addition, attempts to establish this exotic parasite have been made at several locations in Southern Michigan.

PARASITES AND PREDATORS OF THE CEREAL LEAF BEETLE

In 1963 Agricultural Research Service entomologists began search for parasites of the CLB in Europe, and the first shipments of parasites were made from Europe in 1964 (R. I. Sailer, unpublished report, 1967).

Egg Parasites

Anaphes flavipes (Foerster), and other forms of *Anaphes* with which this paper deals, are mymarid egg parasites of the CLB. *Anaphes flavipes* also is known to attack the eggs of *Oulema gallaeciana* Heyd. and has been found in Italy, Spain, Yugoslavia, France, and Germany (R. I. Sailer, unpublished report, 1967).

It appears that two species of *Anaphes* may be involved in the host-parasite relationship, as evidenced by unsuccessful crosses between the strains of *Anaphes* from France and Yugoslavia made by workers at the Agricultural Research Service Biological Control Station at Niles, Michigan, where *Anaphes* is mass-cultured for field release (Moorehead, personal communication). The workers at Niles were unable to obtain females from crosses of French males with Yugoslavian females and vice versa (*Anaphes* will produce only male progeny if unmated).

Pattasson valkenbergica Soyka was reported by Bakkendorf (1964) to have been recovered from the eggs of Oulema lichenis Voet (=gallaeciana) at Tours, France in 1963. Since Anaphes parasitizes O. gallaeciana eggs, it is possible that P. valkenbergica may attack CLB eggs, although this has not yet been proven. In addition, at least one and possibly two species of Trichogramma that parasitize CLB eggs occur naturally in Berrien County, Michigan.

Larval Parasites

The following is a summary of the larval parasites of Anaphes flavipes discussed by R. I. Sailer (unpublished report, 1967). Lemophagus curtus Townes, an ichneumonid originally called Hypersoter sp., has been found in Italy, France, Yugoslavia, Germany, Denmark, and Sweden. Its facultative diapause facilitates lab rearing, since a continuous supply of parasites can be maintained. Diapause may furnish a good means of stock-piling Lemophagus for release the following spring.

Another ichneumonid larval parasite is Tersilochus carinifer Thompson, which has been collected in Yugoslavia, Italy, France, Germany, and Scandinavia. A different but closely related species has been collected in Denmark. Tersilochus has an obligatory diapause.

Tetrastichus julis (Walker) is another hymenopteran larval parasite of the CLB. This eulophid has been found at all localities in Europe where CLB larvae have been collected

and reared. It appears to be more important in the northern part of its range than is Tersilochus. It has a facultative diapause.

A tachinid, Meigenia mutabilis Fall has been reported by Petar Bjegovic (unpublished report, 1966) to emerge from CLB pupal cells in Yugoslavia. This fly parasitized a number of chrysomelid larvae in addition to the CLB.

Hyperparasites

According to R. I. Sailer (unpublished report, 1967), Mesochorus is a hyperparasite known to attack Tersilochus and possibly Lemophagus.

Predators

Known predators of the CLB include Nabis feroides Romane, a hemipteran in the family Nabidae. In Europe prior to the build-up of Anaphes in the last part of May, 1966, Nabis feroides appeared to be very effective against CLB eggs (Petar Bjegovic, unpublished report, 1966). In 1967, however, very few N. feroides could be found (Dysart, personal communication).

Another nabid, Nabis ferus L., is known to be a predator of CLB eggs and larvae. N. ferus is widespread in northern Europe, but does not appear to be abundant enough to reduce populations of the CLB (Petar Bjegovic, unpublished report, 1966).

According to Castro (1964), the lady beetle Coleomegilla maculata lengi Timberlake, feeds on the eggs of the CLB in Michigan in early spring when its preferred hosts (aphids) are not available. Other coccinellids have been seen in the field during the time that C. maculata lengi was active, but their roles as predators of the CLB are unknown. Castro also observed an unidentified pentatomid kill a CLB larva, a carabid beetle attack and kill adult CLBs on the ground, and a cantharid which attacked both adults and larvae.

MYMARIDS IN BIOLOGICAL CONTROL

Although not a mymarid, one of the earliest trials of an egg parasite in biological control was the use of Trichogramma pretiosa (Riley) against the currant sawfly, Nematus ventricosus [=ribesii (Scopoli)]. In 1882, a large number of leaves bearing parasitized eggs of the currant sawfly were collected and mailed to entomologists in various parts of the U.S.A. and Canada (Lintner, 1882). The leaves were to be pinned to currant bushes where the currant sawfly eggs are found. Nothing was said of the results of these mailings.

One of the most successful examples of a mymarid used for biological control was the introduction of Anaphoidea nitens Girault into South Africa from Australia to control the eucalyptus snout-beetle, Gonipterus scutellatus Gyllenhali (Tooke, 1953). According to Tooke, the program to control G. scutellatus "may be claimed to be successful, since out of 65 species of eucalyptus originally attacked, only two species, E. viminalis and E. insizwaensis, now suffer severe damage and these only in certain restricted areas." Tooke further states in regard to the use of A. nitens that "no other method of control would have proved so economical. . . ."

According to DeBach (1964), A. nitens was also introduced into New Zealand in 1927 where it gave "substantial" control of G. scutellatus. He also reports that A. nitens gave "substantial" control when introduced into both Kenya and Madagascar. In Mauritius, where this parasite was introduced in 1946, control of G. scutellatus was complete (DeBach, 1964).

Anagrus epos Girault is another example of a mymarid used in biological control, and provides a good example of the importance of a knowledge of an insect's ecology and habits. On grape in Napa and Sonoma Valleys in California, A. epos has provided commercial control of the leafhopper Erythroneura elegantula (Osborn). It was found that in those areas where this insect was not controlling the leafhopper satisfactorily (San Joaquin and Sacramento Valleys of California), there were no wild blackberry plants growing nearby (Doutt and Nakata, 1965). It was found that Anagrus must overwinter in the eggs of the leafhopper Dikrella cruentata Gillette, which is found on wild blackberries. This secondary host, which oviposits all year, is needed since the primary host, E. elegantula, does not oviposit in the winter months.

Another species of Anaphes known to attack insect pests is Anaphes nipponicus Kuwayama, an important egg parasite of Lema oryzae Kuwayama, the rice leaf beetle (Kuwayama, 1932). Muhle and Fröhlich (1953) reported that in Germany

Anaphes brachygaster was found to parasitize the eggs of the weevil Leophloeus tessulatus found on Levisticum officinale, which is grown for its aromatic and medicinal properties. In addition, Anaphes ovijentatus (Crosby and Leonard) attacks the eggs of the mirid Lygus hesperus Knight, according to Romney and Cassidy (1945).

One of the mymarids with the most interesting habits is Caraphractus cinctus Walker, a parasite of dytiscid eggs in Europe (Jackson, 1958). The female swims underwater by rapid vibrations of her wings and oviposits on the dytiscid eggs she finds.

Although there are many known egg parasites, only a very small number of these have been studied in any great detail, and even fewer have been used in biological control. Several of those used in biological control have had rather spectacular results. From these successes, it appears that egg parasites do have great potential in the biological control of pests. But before the potential is realized, much more work and study remains to be done with the known egg parasites as well as with those that will be discovered in the future.

LIFE HISTORY OF ANAPHES

A new generation of Anaphes begins when a female locates a suitable egg and oviposits in it ("stings" it). The female Anaphes walks about tapping her antennae vertically in front until she finds a CLB egg; she then mounts the egg, searches for an appropriate site, and oviposits. While ovipositing, she stands motionless with her antennae held in front of her. Although she may stand on the end of the egg when ovipositing, she normally is situated on the "top" or side of the egg and is oriented parallel with the long axis of the egg. The egg laid by Anaphes flavipes is elongate and semitransparent, with a small "stem" on one end (Fig. 1). Superparasitism of CLB eggs is normal, usually with from one to three parasites developing successfully from one CLB egg.

At room temperature (70 - 80° F), the earliest a parasitized egg can be identified is from 2 to 3 days after the CLB eggs have been "stung." At this time, under the microscope, from one to several larvae, which appear as "spots" slightly darker than the surrounding yolk may be seen. Parasitized eggs can more definitely be identified at pupation, when compound eyes are formed from 6 to 8 days after parasitization, depending on temperature. By the

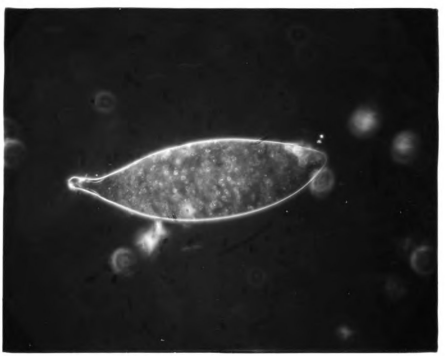


Figure 1 - The egg of Anaphes flavipes, 250x.
(Photograph by Richard Snider, Department of Entomology, Michigan State University)

eighth to tenth day, the black pupae of the parasite are clearly visible; in fact, to the naked eye the entire egg appears black except for some small orange spots of meconium. Depending on the temperature, adults emerge on the ninth to thirteenth day after the parent parasites are placed on the eggs.

Parasites can be reared the year-round in the laboratory without interruption because laboratory conditions have not induced diapause. It is not known whether Anaphes overwinters as an adult, as an immature within the egg of the CLB, or within the egg of some alternate host.

In Europe, parasitized CLB eggs have been found as early as 20 May, and adult Anaphes have been found as early as 17 May (R. I. Sailer, unpublished report, 1967). Early in the season, eggs parasitized by Anaphes are difficult to find, but by the end of the CLB oviposition period the parasitization rate comes close to 100% in some fields (Petar Bjegovic, unpublished report, 1966).

At present there are Anaphes cultures from various parts of Europe including Italy, France, and Yugoslavia (R. I. Sailer, unpublished report, 1967). The original Anaphes culture came to the United States from Paris, France in 1965 (R. I. Sailer, unpublished report, 1967). This culture and the 1966 Yugoslavian culture are the two that have been used for all the work reported here. The original Yugoslavian culture was sent to the United States in 1966

through the cooperative efforts of Dr. Petar Bjegovic of the Laboratory for Biological Control, Banatska, Zemun, Yugoslavia, Dr. J. W. Butcher (Entomology Department, M.S.U.), Dr. R. I. Sailer (U.S.D.A., formerly head of the European Parasite Laboratory), and M. H. Brunson (Insect Identification and Parasite Introduction Research Branch, U.S.D.A., Moorestown, N.J.).

DEVELOPMENT OF MASS CULTURING

Objectives and Review

According to DeBach (1964), "the goal of a mass-culture programme is to produce with minimum man hours and space the maximum number of fertile females of an entomophagous species in as short a time and as inexpensively as possible." In the mass culturing of insects, it is necessary to gain as much knowledge about both the host and parasite as quickly as possible. This information ranges from mating behavior under laboratory conditions to fecundity and longevity of the insect cultured (both the host and entomophagous insect). Factors such as superparasitism and cannibalism by either the host or entomophagous insect must be taken into consideration.

The ideal host used in mass culturing should be readily accepted by the entomophagous insect to be reared (Debach, 1964). The host should: (1) be readily cultured, (2) have a rapid rate of increase, (3) present no serious mating problems, (4) not give off any undesirable by-products, (5) be a general feeder, and (6) be highly resistant to disease.

According to Flanders (1949) the actual procedure in culturing the insects includes the segregation of operations,

operational factors, and handling of the populations. In the rearing of Anaphes the preparation of host-supporting medium and propagation of the host have been kept segregated from the culturing of Anaphes, either by rearing Anaphes in a different building or in a different room than that used for rearing the host supporting medium and the host. Operational factors, such as temperature and humidity, have been arranged so that it is possible to complete a generation approximately every 10 days.

Mass rearing Anaphes could possibly be accomplished using either of two basic techniques: (1) removing CLB eggs from plants and confining them with Anaphes or (2) parasitizing CLB eggs on the plants upon which they were laid.

The following section will describe the current method of rearing Anaphes as used at the Agricultural Research Service Biological Control Station at Niles, Michigan, which is responsible for mass rearing Anaphes for field release. The method used at Niles for rearing Anaphes involves the removal of CLB eggs from plants.

Mass Culturing Used at Niles in 1967

The eggs used for rearing Anaphes are generally a minimum of 24 hours old. While on the plants, the eggs are dipped in a suspension of Captan (.1%), a fungicide which effectively retards the growth of fungus on the eggs while they are being stored and during the time that the eggs contain developing Anaphes. Once the eggs have been treated

with fungicide, each individual egg is removed by placing a dissecting needle next to the egg, gently lifting it from the plant, and placing the egg on a glass coverslip (35 mm x 30 mm). Normally 150 eggs are placed on each coverslip in this manner. The coverslip, which is within a small (50 mm x 12 mm) petri dish, has petroleum jelly on the bottom of it so that the coverslip will not move about in the dish while the eggs are being transferred. The petri dish has moist filter paper on the bottom and a tight fitting top that helps keep moisture within the dish. Currently, Niles is developing a technique for egg removal from plants which eliminates the need for hand picking. This particular technique not only removes CLB eggs from plants but it also "desticks" the eggs.

When handling the parasites, a Thermolyne Laboratory Light, with a fluorescent tube mounted behind a plate of transluscent plastic, is used in a partially darkened room in which parasites are handled. The photopositive parasites are attracted to the light and can be easily handled with a small brush or vacuum-operated collecting pipette.

The physical handling of Anaphes originally was done with a fine camel hair brush, but a soft sable hair brush is now used. The parasites become entangled in the bristles and in this manner can easily be transferred. A microaspirator is used also. The microaspirator consists of a piece of tygon tubing connected to a disposable pipette

(15 cm long). The end of the tubing that fits into the pipette is covered with a piece of nylon organdy cloth to keep parasites from being sucked into the vacuum generator, which is a milking machine. The parasites can mate within the pipette, and when females are wanted, the pipette's open end is oriented toward the light, and as the parasites leave the pipette they are picked up with the sable hair brush.

Presently at Niles, the parasites receive no food or water before being placed on the eggs to be parasitized, and they remain there until they die. They receive water from the moist filter paper in the dish with the eggs to be parasitized.

At Niles, unparasitized eggs are stored at 40° F until they are needed. At present, Niles does not use eggs that have been stored for more than 2 months, and they prefer to use eggs that have been stored for no more than 6 weeks. Older eggs are not used because their acceptability to Anaphes is reduced too greatly.

The parasites may also be stored at 40° F in the black pupal stage which occurs from 1 to 2 days before the adult parasites emerge. Niles prefers to store Anaphes for no more than 2 weeks. This is because there appears to be a decrease in the activity of adult parasites the longer they are stored as black pupae.

Use of Hospital Talc on Fresh Eggs

In the laboratory rearing of Anaphes, when 1 or 2-day-old eggs that have been removed from plants are exposed to Anaphes for parasitization, the parasites may become permanently stuck to the eggs. Because of the stickiness of the eggs, tests were conducted using eggs treated with hospital talc in an attempt to eliminate the problem.

In the first test, 200 eggs were used of which 100 were treated with talc. All eggs were placed in the same dish and then 28 female and 14 male Anaphes were confined with the eggs.

When on the talc-treated eggs the parasites continued to get stuck, but they managed to free themselves. After leaving a cluster of talc-covered eggs, they seemed to lose balance for a short period of time and sometimes fell on their backs.

The parasites were left in the dish until they died. Of the dead parasites, two males were stuck to talc-treated eggs and 23 parasites, 11 of which were females, were stuck to the untreated eggs. The remaining 17 females were not in contact with the eggs. Before the parasites had completed development, the dish was accidentally destroyed.

In the next test, 20 untreated eggs and 15 talc-treated eggs were placed in the same dish and one female parasite was added and observed. When she contacted the talc-covered eggs, she would get off the slide and clean

herself; after contacting the talc she sometimes staggered and even fell on her back, often having trouble righting herself. Probably the imbalance was caused by talc particles attached to the insect's tarsi, but it is possible some form of toxicity might have been involved. Twenty parasites emerged from the untreated eggs whereas nothing developed from those treated with talc, indicating that parasitization of talc-treated eggs was not accomplished.

Tests using talc were discontinued for several reasons. The activity of the parasites appeared to be adversely affected by the talc and it was found that allowing the eggs to air dry for several hours before parasitization would sufficiently decrease the stickiness of the eggs, especially when they had been stored at 40° F.

Daily Feeding of Anaphes to Increase Progeny per Female

To test the possibility that the number of eggs parasitized by a given number of female Anaphes could be increased by feeding and watering the parasites every day, the following experiment was conducted. On day one, five French female parasites were placed on each of 50 test and control eggs. The following day, the live females on the test eggs were removed, placed in a dish with moist filter paper and honey for 1 hour and then placed on a fresh group of 50 eggs. This procedure was continued until all the parasites were dead. The females with the check eggs were

left there until they died. The eggs used in the tests were from 1 to 2-days old and never stored for more than 4 days at 40° F before use.

Tests 6 to 9 were begun the same day and the females used emerged the same day; thus, only one group of 50 eggs was used as check for the four tests. The results of all the tests are presented in Table 1.

There is an increase in the total number of eggs parasitized and progeny produced but it appears that the increase is not sufficient to warrant the extra work. Since there are fewer eggs parasitized each succeeding day, and larger numbers of eggs are unparasitized when compared with the use of fresh females on the eggs, this technique would probably be most valuable when there is a shortage of female Anaphes and an abundance of eggs.

Tests 1, 2, 4, 7, 10, and 11 indicate that it is only necessary to mate Anaphes on the first day of testing to obtain an adequate F_1 sex ratio. Tests 6, 8, and 9 appear to indicate the opposite. Since the controls for 6, 8, and 9 had slightly more males than females develop, the unsatisfactory female to male sex ratio of the tests is due to inadequately mated females at the beginning of the tests.

Table 1. The effect of daily feeding and watering of *Anaphes flavipes* and daily transfer to fresh eggs on the number of offspring produced per female *Anaphes*

Test	Day	No. Eggs	No. BP[a]	No. *Anaphes* Used	Progeny ♀	Progeny ♂	Percent Parasitization
1	1	50	40	5	74	16	80
	2	50	24	5	34	3	48
	3	50	7	4	9	2	14
Total		150	71		117	21	47
Check[b]		50	38	5	68	7	76
2	1	50	34	5	58	13	68
	2	50	23	5	38	6	46
	3	50	15	4	21	8	30
Total		150	72		117	27	48
Check[b]		50	34	5	51	7	68
3	1	50	20	3	42	16	40
Check[b]		50	7	3	9	2	14
4	1	50	36	6	69	8	72
	2	50	14	4	15	2	28
	3	50	0	2	0	0	0
Total		150	50		84	10	33
Check[b]		50	31	6	50	2	62
5	1	50	33	5	36	45	66
	2	50	0	5	0	0	0
	3	50	0	1	0	0	0
Total		150	33		36	45	22
Check[b]		50	33	5	10	67	66
6	1	50	17	5	9	8	34
	2	50	5	4	4	3	10
	3	50	4	3	3	4	8
Total		150	26		16	15	17
Check[b]		50	28	5	22	24	56

Table 1--Continued

Test	Day	No. Eggs	No. BP[a]	No. Anaphes Used	Progeny ♀	Progeny ♂	Percent Parasitization
7	1	50	28	5	25	6	56
	2	50	5	4	9	2	10
	3	50	0	2	0	0	0
Total		150	33		34	8	22
Check[b]		50	28	5	22	24	56
8	1	50	38	5	25	35	76
	2	50	14	5	7	15	28
	3	50	2	4	0	3	4
Total		150	54		32	53	36
Check[b]		50	28	5	22	24	56
9	1	50	11	5	0	6	22
	2	50	5	4	7	4	10
	3	50	4	2	0	7	8
Total		150	20		7	17	13
Check[b]		50	28	5	22	24	56
10	1	50	34	5	58	11	68
	2	50	7	3	12	1	14
	3	50	0	1	0	0	0
Total		150	41		70	12	27
Check[b]		50	23	5	35	6	46
11	1	50	15	5	17	2	30
	2	50	1	4	2	0	2
Total		100	16		19	2	16
Check[b]		50	39	5	61	10	78

[a]BP = the number of parasitized eggs that developed black pupae.

[b]Check - the parasites were left with the original 50 eggs until the parasites died.

Collecting Container

An apparatus for automatically collecting Anaphes upon emergence from petri dishes was devised (Fig. 2). The apparatus consisted of a plastic funnel (inside diameter of 91 mm) with all but the neck painted black on the outside. The neck was removed and inserted near the open end of the funnel at a 45° angle. This was done because an earlier version of the device utilizing a vertical neck did not adequately collect the parasites. Possibly the movement of Anaphes within the vertical collecting device caused interference to such an extent that they fell back into the petri dish.

Into the neck, held in place by friction, was inserted the widest end of a disposable glass pipette (15 cm long). The narrow end of the pipette was closed with a small piece of cork. A piece of black cloth was placed at an angle inside the funnel so that the highest point was above the exit opening where the neck inserted into the funnel.

The glass slides with parasitized eggs in the black pupal stage were put into a plastic disposable petri dish (89 mm in diameter) that contained moist filter paper. The funnel was placed over the petri dish and taped down. The parasites that emerged were collected in the pipette. Table 2 presents the results of collecting Anaphes with this device. Most of the parasitized eggs that had no parasites

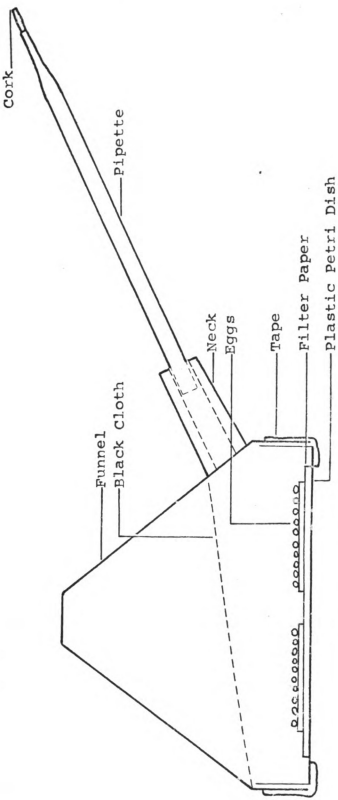


Figure 2 - Container used for collecting newly emerged *Anaphes*.

Table 2. The efficiency of the Anaphes flavipes self-collecting apparatus

Test No.	No. BP[a]	No. Anaphes in Pipette ♀	No. Anaphes in Pipette ♂	No. Anaphes in Dish ♀	No. Anaphes in Dish ♂	No. of Dead Anaphes in Dish ♀	No. of Dead Anaphes in Dish ♂	Emergence[b]
1	101	122	83	18	29	0	0	93
2	92	116	18	7	5	13	4	75
3	64	58	7	3	3	1	1	41
4	126	145	18	9	13	12	0	100[c]
5	32	39	4	0	2	2	0	20
6	34	47	6	2	0	3	4	29
7	61	81	6	4	13	3	0	50
8	60	71	19	5	3	1	0	50
Total	570	679	161	48	68	35	9	458

[a]BP = the number of parasitized CLB eggs with black pupae.

[b]The number of parasitized eggs that had parasites emerge.

[c]The balance of parasitized eggs were removed before they could emerge.

emerge were heavily covered with fungus. Ninety-three percent of the females and 70 percent of the males that emerged were recovered in the pipette.

The male parasites are not as easily collected as are the females using this apparatus. Possibly the males are not as photopositive as the females or they may tend to stay close to the parasitized eggs so they can mate with the emerging females.

Several tests were conducted to discover whether mating occurs within the collecting apparatus. The Anaphes

used in the test group were removed from the pipette and put directly on the eggs. The controls consisted of one or a combination of the following: Anaphes taken from the pipette, placed in a mating capsule, and then put on eggs; or Anaphes taken from a 50 x 12 mm petri dish, placed in a mating capsule, and then put on eggs. The Anaphes placed in mating capsules were left there for 1/2 to 1 hour. The results of these tests are presented in Table 3.

Table 3. The percent female offspring from Anaphes flavipes collected and mated in the collecting apparatus

Test Directly from Pipette		Control Pipette to Capsule		Control[a] Dish to Capsule
Sex Ratio of Parents in Pipette (♀:♂)	% Fem. Offspring	Sex Ratio of Parents in Pipette (♀:♂)	% Fem. Offspring	% Fem. Offspring
8:1	78.5	7:1	80.3	83.9
14:1	75.3	...	....	0.0
6:1	28.3	4:1	0.0	...
8:1	35.2	7:1	67.7	...
2:1	78.8	1:1	82.3	...
2:1	84.8	1:1	82.3	...
11.5:1	52.8	10:1	90.0	...

[a]The sex ratio used for these controls was not recorded.

The results of the tests indicate that mating does occur within the collecting device, although it is unknown whether it occurs within the dish, the pipette, or both. The percentage of females to develop from adults placed on eggs directly from the pipette is similar to the controls for tests 1, 5, and 6. The controls for tests 2 and 3 had no females develop even though females (75.3% for test 2 and 28.3% for test 3) developed from eggs on which adults were placed directly from the collecting pipette. It appears that not only is a large number of the emerging females collected by this device, but that sufficient mating can occur within the pipette to preclude further measures to insure mating.

Parasitization in the Dark

Several tests were conducted to discover whether Anaphes will parasitize CLB eggs in total darkness. A female and three male Anaphes were placed in a dish containing 20 eggs which was then covered with a cloth and placed in a closed cabinet, to keep light from reaching the parasites and eggs. The controls consisted of one female and three male French Anaphes on 20 eggs in daylight. No parasites developed in the eggs that had been in darkness whereas 20 parasites were obtained from the eggs used as controls. The age of the eggs used in both the test and control groups was not known but both were from the same group of eggs so the age of the test and control eggs was the same.

Because of the inadequate number of parasites available for the previous test, Test 2 was conducted, using pots with glass globes and eggs left on the plants. Although the age of the eggs was not known, it was the same for both the test and control groups. The control was left with access to daylight while the test group was covered with two layers of black cloth and placed in a cabinet. The space between the closed doors was sealed to insure total darkness within the cabinet. The cabinet was opened once, 2 days later, long enough to water the plants. Eighteen female and five male Anaphes (French) had been placed in each of the containers at the beginning of the test. The results are presented in Table 4.

Table 4. The parasitization of cereal leaf beetle eggs by Anaphes flavipes in total darkness (Test 2)

Test	No. Eggs	No. Eggs Parasitized	Percent Parasitized	No. Females Emerged	No. Males Emerged
Darkness	104	15	14.4	8	6
Daylight	92	37	40.2	45	30

Although not conclusive, the tests indicate that Anaphes does not parasitize as effectively in total darkness as under normal photoperiods. Some of the parasitization under "total darkness" for Test 2 could have taken place while the parasites were being added and before the containers were placed in total darkness and/or when the plants were watered.

Containers Used for Mass Culturing Anaphes while CLB Eggs Are on the Plants

To save time in rearing Anaphes by leaving CLB eggs on the plants (and thereby avoiding hand picking), a number of rearing containers were tested. The procedures used with the various containers were similar. Wheat or barley was planted in soil placed in the container. Once the plants were tall enough (3 or 4 inches), beetles were confined on them and allowed to oviposit. Before the beetles were placed in the container, the soil was covered with white sand, facilitating removal of the beetles. After oviposition, the beetles were removed, the eggs were counted, and parasites in a gelatin capsule were dropped into the container. After 7 or 8 days, the plants were removed one by one and those eggs that did not hatch were checked for parasitization. The eggs were allowed to develop, and those with black pupae were counted. The parasites were counted and sexed on emergence.

The first container used was a clear plastic box, 14 x 10.5 x 6.5 inches (Figs. 3 and 5). In the cover, also made of clear plastic, a hole approximately 10 x 8 inches had been cut and covered with a piece of fine white cloth to keep parasites from escaping (the top was taped down during usage). The bottom of the plastic container had six slits approximately 1/6 of an inch wide equally spaced along the length of the bottom to allow water into the soil within the plastic container. In Table 5 this container is referred to as the "large" container.

Another plastic box used was similar to the above in that it was rectangular and the plants were allowed to grow in it (Figs.4 and 5). The bottom was a gray plastic flat (8.5 x 5.5 x 2.5 inches) while the top consisted of a plexiglas box that fit inside the bottom. The top had three, 2-inch holes (one on the top and one on each end) covered with fine cloth to keep parasites from escaping. The top was placed over the plants and pushed into the soil of the plastic container, forming a tight seal. Beetles were placed in the plastic box by dropping them through the hole in the top. They were removed with an aspirator.

Parasites were released in the small plastic box by dropping a gelatin capsule containing the parasites through the hole in the top which was thereafter covered with the fine cloth. In Table 5, this small plastic box is referred to as the "small" container. Although the dimensions of

Figure 3 - The "large" mass culture box without its top.

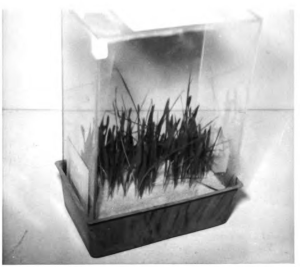


Figure 4 - The "small" mass culture box.

Table 5. A comparison of three different mass culture containers, using French (F) and Yugoslavian (Y) strains of Anaphes

Type Container	No. CLB Eggs	Egg Age (days)	No. Anaphes and Strain	No. CLB Eggs/♀ Parasite	No. Eggs With Black Pupae	No. F_1 ♀	No. F_1 ♂	No. Eggs With BP/♀ Used[a]	Mean No. F_1[b]	Percent Parasitization[c]
Large	369	2	13 Y	28	103	100	76	7.92	13.5	28
Large	790	3	60 Y	13	354	369	232	5.90	10.0	45
Large	599	..[d]	60 Y	10	402	640[e]	..	6.70	10.7	67
Large	700	4	57 F	12	281	217	261	4.92	8.4	40
Large	614	3	18 F	34	205	329	84	11.38	23.0	33
Large	460	..[d]	18 F	26	161	72	285	8.94	19.8	35
Large	200	3	20 F	10	142	317	52	7.10	18.5	71
Large	302	3	13 F	23	59	100	23	4.53	9.5	20
Large	191	3	20 F	10	84	145	27	4.20	8.6	44
Large	230	3	15 F	15	34	36	7	2.26	2.9	15
Large	140	..[d]	25 F	6	106	109	88	4.24	7.9	76
Large	455	..[d]	26 F	18	100	168[e]	...	3.84	6.5	22
Small	124	3	15 Y	8	21	15	12	1.40	1.8	17
Small	238	3	14 Y	17	75	...[f]	...	5.35	...	32
Small	152	2	15 Y	10	82	...[f]	...	5.46	...	54
Small	583	2	33 Y	18	36	71	12	1.09	2.5	6
Small	243	1	16 Y	15	92	20[g]	3	5.75	...	38
Small	347	2	23 Y	15	130	31[g]	7	5.65	...	37
Small	184	1	9 Y	20	19	18	4	2.11	...	10
Small	460	3	50 F	9	180	147	76	3.60	4.5	39
Small	142	3	25 F	6	82	...[f]	...	3.28	...	58
Small	101	3	10 F	10	66	94	43	6.60	13.7	65
Small	134	2	10 F	13	29	27	7	2.90	3.4	22
Small	190	1	15 F	13	29	18	21	1.93	2.6	15
Small	286	1	18 F	16	57	10[g]	1	3.16	...	20
Small	163	1	15 F	11	26	5	40	1.73	3.0	16
Small	316	2	31 F	10	146	2[g]	2	4.71	...	46
Small	384	1	26 F	15	158	112[g]	22	6.07	...	41
Small	164	1	10 F	16	51	63	28	5.10	9.1	31
Globe	418	3	30 F	14	149	58[g]	14	4.96	...	36
Globe	278	3	20 F	14	157	121	35	7.85	...	57
Globe	95	3	10 F	10	54	43	18	5.40	6.1	64
Globe	110	..[d]	11 F	10	...[d]	110[e]	...	....	10.0	..
Globe	74	..[d]	5 F	15	...[d]	110[e]	...	....	22.0	..
Globe	58	..[d]	3 F	19	...[d]	34[e]	...	....	11.0	..

[a] BP = the number of parasitized eggs that developed black pupae.

[b] The mean number of F_1 parasites to emerge per female parent used in the container.

[c] The percent parasitization is obtained by dividing the total number of CLB eggs by the number of CLB eggs with parasite black pupae.

[d] Age unknown. [e] These offspring were not sexed. [f] Emergence not observed.

[g] Failure to obtain better emergence was due to long term storage of the parasitized eggs at 40° F.

this and the large plastic box are different, the area enclosed by both boxes is similar.

This small plastic box has advantages over the first one in that a tight fit between the top and bottom is obtained without having to tape the top down and it is smaller and easier to handle. With the present cages used for rearing the CLB, the small plastic box is more convenient, since it can be placed within the beetle oviposition cages.

In addition to the above plastic boxes, lantern globe-covered plastic pots were used (Fig. 5). Beetles were either confined on the plants using a glass globe or the pots were placed in oviposition cages. The soil was covered with white sand before oviposition. After oviposition, the parasites (French) were placed in a gelatin capsule and dropped into the container through the globe's top which was then covered with fine cloth held in place by a rubber band. The glass globe was taped to the plastic pot to keep it from accidently being knocked off the pot.

The effectiveness of the small plastic boxes versus the large plastic boxes without regard to the strain of Anaphes used was tested in terms of the number of parasitized eggs to develop per female Anaphes used. The Mann-Whitney U Test was used for analysis. Since the mass-culturing tests were conducted at different times, it is assumed that any generation of parasites is as good as the

Figure 5 - The mass culture containers: from left to right they are the "large," globe, and "small."

preceding and following generations. There is no reason to suspect that one generation of parasite is better than another unless separated by many generations. In such a situation there could be differences due to inbreeding and/or selection of a laboratory-favored gene pool. The same assumption has been made in regard to the CLB eggs that were used in the various trials. The analysis of the data revealed that there is a difference between the plastic boxes in favor of the large plastic boxes at the 5% level of significance but not at the 1% level.

The Mann-Whitney Test also was used to test for any significant difference between the Yugoslavian and French strains of Anaphes that were used in the large and small plastic boxes. At the 5% and 1% levels of significance the analysis revealed no differences in the strains in terms of the number of CLB eggs parasitized per female used.

In addition to the above tests, the Mann-Whitney Test was used to test the following parasite strain-plastic box combinations: (1) Yugoslavian strain of Anaphes in the large box vs. the Yugoslavian strain in the small box; (2) French strain of Anaphes in the large box vs. the French strain of Anaphes in the small box; (3) French strain in the large box vs. the Yugoslavian strain in the large box; (4) French strain in the small box vs. the Yugoslavian strain in the small box; (5) French strain in large box vs. the

Yugoslavian strain in small box; (6) French strain in small box vs. the Yugoslavian strain in the large box.

The tests showed that at the 5% level of significance there was no difference between the pairs that were tested except the Yugoslavian strain in the two different plastic boxes (1) favoring the large box. The Yugoslavian strain in the large plastic boxes (6) was favored over the French strain in the small plastic boxes.

The use of French Anaphes in lantern globes was tested against the use of the Yugoslavian and the French strain in both the large and small plastic boxes. The use of the lantern globe versus the use of the Yugoslavian strain of Anaphes in large plastic boxes was not analyzed because there were not enough samples to conduct the analysis.

According to the Mann-Whitney Test, at the 5% level of significance there was no difference in the use of the French Anaphes in the lantern globes and the use of the French strain in either of the other plastic boxes. This is true also in the comparison with the Yugoslavian strain in the small plastic boxes. Visual examination of the data indicated that there was no difference for the comparison of the French Anaphes in the lantern globe and the Yugoslavian strain in the large plastic boxes.

Table 6 includes the results of rearing both the Yugoslavian and French strains of Anaphes by the method that is currently in use at Niles; that is, in 50 x 12 mm petri dishes with tight-fitting lids. There are a few differences however. Niles uses 150 eggs and 10 female parasites per dish whereas the number of female parasites and the number of eggs used per dish in Table 6 varies from dish to dish. The data of Table 6 will be used to compare the effectiveness of the two basic methods of mass rearing Anaphes.

The Mann-Whitney Test, indicated no difference between the Yugoslavian Anaphes and the French Anaphes reared in the 50 x 12 mm petri dishes in terms of the number of parasitized eggs per female parasite used. The same analysis also indicated that there was no difference between the use of the plastic boxes and petri dishes. Furthermore, the effectiveness of the Yugoslavian strain in the plastic boxes was compared with the Yugoslavian strain in the petri dishes as well as the effectivenss of the French Anaphes in the plastic boxes compared with the French Anaphes in the petri dishes. The Mann-Whitney Test indicated no differences in the comparisons.

Discussion

Although it is possible to increase the number of parasitized eggs per Anaphes female by putting them on fresh eggs each day, the effort does not seem to be warranted on the basis of the number of eggs used and wasted, especially

Table 6. A comparison of rearing both Yugoslavian and French strains of Anaphes in petri dishes

Rank No.	The Number of Parasitized Eggs Per Tested Female of the Specified Strain	
	French	Yugoslavia
1	.78	.82
2	1.50	.83
3	2.00	1.16
4	2.14	1.33
5	2.33	1.42
6	2.40	1.46
7	2.53	2.20
8	3.50	2.20
9	3.50	3.14
10	4.13	3.25
11	4.20	3.80
12	4.36	3.80
13	4.40	4.14
14	4.60	4.84
15	5.10	5.00
16	6.40	5.40
17	6.40	6.00
18	6.60	6.16
19	6.80	7.00
20	7.00	7.33
21	7.20	12.00
22	8.00	

after the second day when very few eggs are parasitized. The only time this technique might be of value is when there are very few female Anaphes and an abundance of eggs.

Hospital talc decreases stickiness of fresh eggs to parasites but it is not efficient since it causes erratic parasite behavior; i.e., they may stagger and fall on their backs. Furthermore, air drying eggs for several hours before usage decreases stickiness sufficiently.

The pros and cons associated with both techniques of rearing Anaphes (removing eggs vs. leaving them on plants) are numerous. The use of the plastic boxes or globes has the advantage that it is not necessary to handle the eggs. There appears to be no great problem in introducing parasites to the eggs with the use of the plastic boxes or globes. It is also possible that with the use of the plastic boxes, feeding and watering of emerging parasites would be no problem since they may get what they need from within the boxes. Also, parasites probably do not become stuck to eggs as readily since they will not come into contact with the eggs as often as is the case within the petri dishes where the eggs are concentrated. In the case of the field release of parasitized eggs on plants, one would only need to place the leaves bearing the pupae into the field.

On the other hand, there are drawbacks to the use of the plastic boxes and globes for mass culturing. Although the boxes and globes appear to be as efficient as the petri dishes in terms of the number of parasitized eggs to develop per female used, they take up much more space than do the petri dishes now in use. It is much more difficult to make accurate egg counts using the plastic boxes or globes. It would be necessary to have a table that would give an estimate of the number of eggs laid by a given number of beetles of a given age for a given length of time. With such a table, estimating the number of eggs within a plastic

box or globe would not be difficult. Related to this is knowing how many parasites to introduce into the box, but this could be overcome once a table as mentioned above is developed.

One of the biggest drawbacks to the use of the petri dishes (the technique currently used for mass culturing Anaphes) is the great length of time necessary to remove the eggs from the plants by hand prior to parasitization. Thus, with the two techniques, the one best suited for use will be determined by the time and space requirements and by the manpower available. The development of a technique for non-manual removal of eggs from leaves would make the petri dish method (removal of eggs from plants) far superior to the use of the much larger mass rearing boxes or globes.

Currently, using either the plastic boxes or globes or the petri dishes for rearing Anaphes, there are problems in storing both parasitized and unparasitized eggs. Fungus has been one of the big problems in the storage of CLB eggs.

At present, it is not known how Anaphes overwinters: if this can be determined, the problems involved in storage of Anaphes may be greatly diminished. It must be learned whether the parasites overwinter as adults or immatures and whether or not an intermediate host is involved.

STORAGE OF UNPARASITIZED EGGS

CLB egg storage is important in the mass culture of Anaphes for several reasons. Anaphes develops in approximately 10 days; thus, to maintain an active culture, a continuous egg supply is needed. Since there may be fluctuations in the production of CLB eggs, there are times when sufficient fresh eggs are not available for the continuous rearing of Anaphes. Stored eggs must be used during these times until beetle oviposition increases.

When mass production begins, a large number of eggs must be available to build up the parasite population. In most instances, this means stockpiling the unstung CLB eggs at least a month before the actual parasite population build-up. A rapid increase in the parasite population can be easily achieved when there is a sufficiently large number of eggs with which to work rather than having to wait for the day-to-day production of fresh eggs.

Storage over Water

During the storage of eggs on glass slides or glass coverslips desiccation may occur if enough moisture is not maintained within the storage dish. Consequently, the first storage tests involved inverting slides with eggs over water. This was done in the belief that water would condense on the

underside of the slide with the eggs, thus providing sufficient moisture for the eggs during storage and eliminating the need for the periodic addition of moisture to storage dishes.

In Test 1, 576 unparasitized CLB eggs were put into storage at 40° F. The eggs were placed on slides that were inverted and mounted in the side of a vertical piece of balsa wood (Fig. 6). The mounted slides were then placed in a transparent plastic container (10.5 x 7.5 x 4 inches) with about 1 inch of sterilized distilled water in the bottom and covered with a tight fitting lid. The eggs, not treated with a fungicide, were removed at intervals and subjected to parasitization by Yugoslavian Anaphes. In Test 1g French female Anaphes were used due to the lack of Yugoslavian Anaphes. The results are presented in Table 7 and Fig. 7.

For Test 1g, there was a control consisting of 50, 2-day-old eggs that had been in storage 7 days at 40° F upon which were placed six female French Anaphes. From 34 eggs, 44 parasites emerged. This control indicates that the failure to obtain parasite development from eggs of Test 1g was caused by some factor or factors other than the parasites that were used. There were no other controls. The failure to obtain parasite development in Test 1d were partly caused by the fungal growth since fungus may interfere with oviposition and/or emergence of parasites.

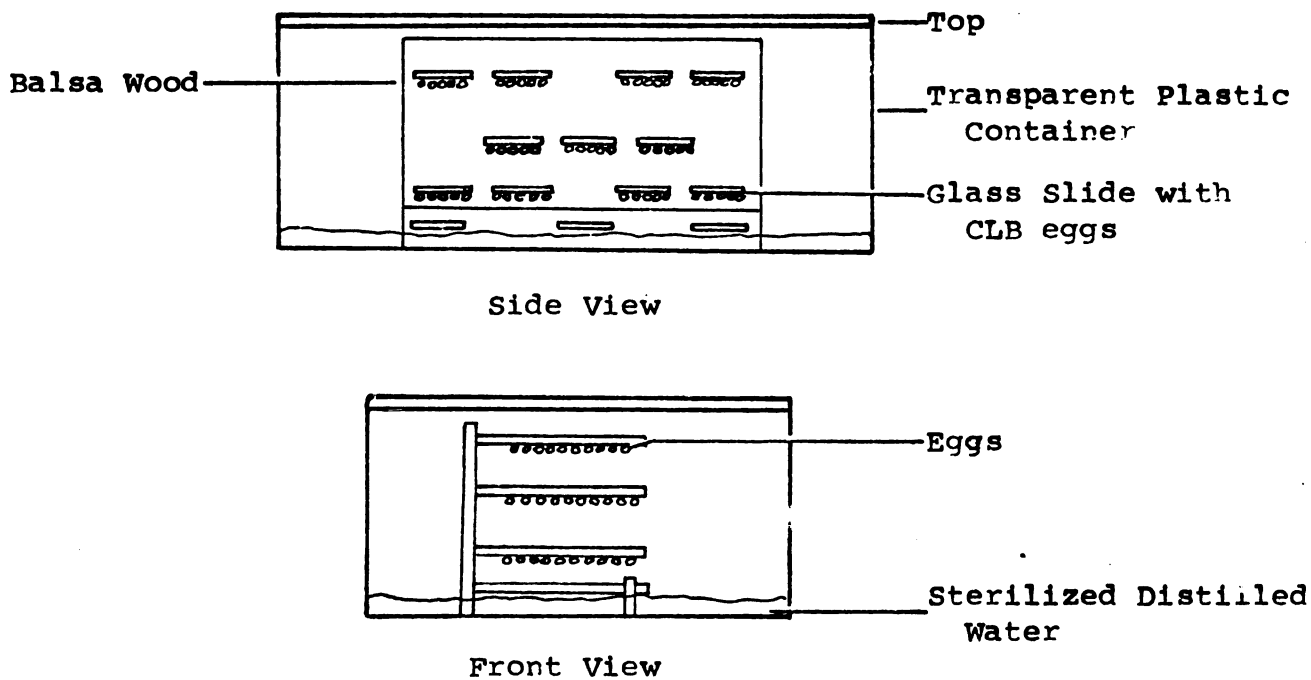


Figure 6 - Container used for storing cereal leaf beetle eggs inverted over water up to 176 days.

Table 7. The influence of the length of cereal leaf beetle egg storage while inverted over water on subsequent parasitization by the Yugoslavian strain of Anaphes (Test 1)

Test No.	No. Eggs	Days Eggs Stored	No. Eggs With Fungus When Taken From Storage	No. ♀ Used	No. BP[a]	No. F_1	Percent Parasitization
1a	74	15	..	10	25	41	33.8
1b	75[b]	23	25	10	20	30	29.9
1c	98	35	7	10	29	37	29.6
1d	75[c]	42	48	10	0	0	00.0
1e	100[d]	69	20	8	16	25	16.3
1f	100[e]	82	21	10	2	2	2.5
1g	54	176	6	6[f]	0	0	00.0

[a]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[b]Eight eggs were not used because of too much fungus.

[c]Seven eggs were removed because of fungus or because they had collapsed.

[d]Two of the eggs were not used because there was too much fungus on them.

[e]Twenty-one eggs were not used (79 used) because of profuse fungus.

[f]The French strain of Anaphes was used.

This set of tests, even without controls, indicates that eggs can be stored for at least 35 days at 40° F while inverted over water with only a slight decrease in the percent parasitization, but after 35 days of storage there is a rather sharp decrease in the number of parasitized eggs.

In Test 2, unparasitized CLB eggs were again inverted over water on glass slides with 50 eggs per slide. They were a maximum of 2-days-old at the time of storage. Six female French Anaphes were used on each set of 50 test and control eggs. The control eggs were either fresh or stored for a maximum of 6 days before use. The results are presented in Table 8 and graphically in Figure 7.

The results of Test 2 indicate a steady decline in acceptability of eggs to parasitization as the length of storage increases, although the results of storage for the first 60 days are within the range of results for the controls of Tests 2a to 2e.

Both sets of tests indicate that CLB egg storage by inversion of eggs over water works well for the first 30 to 60 days. But fungicide treatment should be given before storage of the eggs if this technique is to be used since fungus may pose a problem as storage time increases.

The results of the two tests indicate a difference between the Yugoslavian and French strain, and their acceptance of stored CLB eggs. The eggs appear to be less acceptable to the Yugoslavian strain than to the French strain (Fig. 7). But there is a smaller decline in the Yugoslavian parasitization rate than there is for the French strain. Although it is not known whether the observed differences are due to actual differences between the strains or to experimental factors such as the female-to-egg ratio or the

Table 8. The influence of the length of cereal leaf beetle egg storage while inverted over water on subsequent parasitization by the French strain of Anaphes (Test 2)

Test No.	No. Days Stored	No. Eggs		No. BP[a]		No. Offspring				Percent Parasitization	
						Test		Untreated			
		Test	Untreated	Test	Untreated	♀	♂	♀	♂	Test	Untreated
2a	35	50	75	35	48	52	23	86	19	70	64
2b	48	50	50	26	39	40	12	66	32	52	78
2c	62	50	50	24	24	28	10	31	14	48	48
2d	78	50	50	13	29	18	6	44	14	26	58
2e	100	50	50	2	36	3	1	27	34	4	72

[a]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

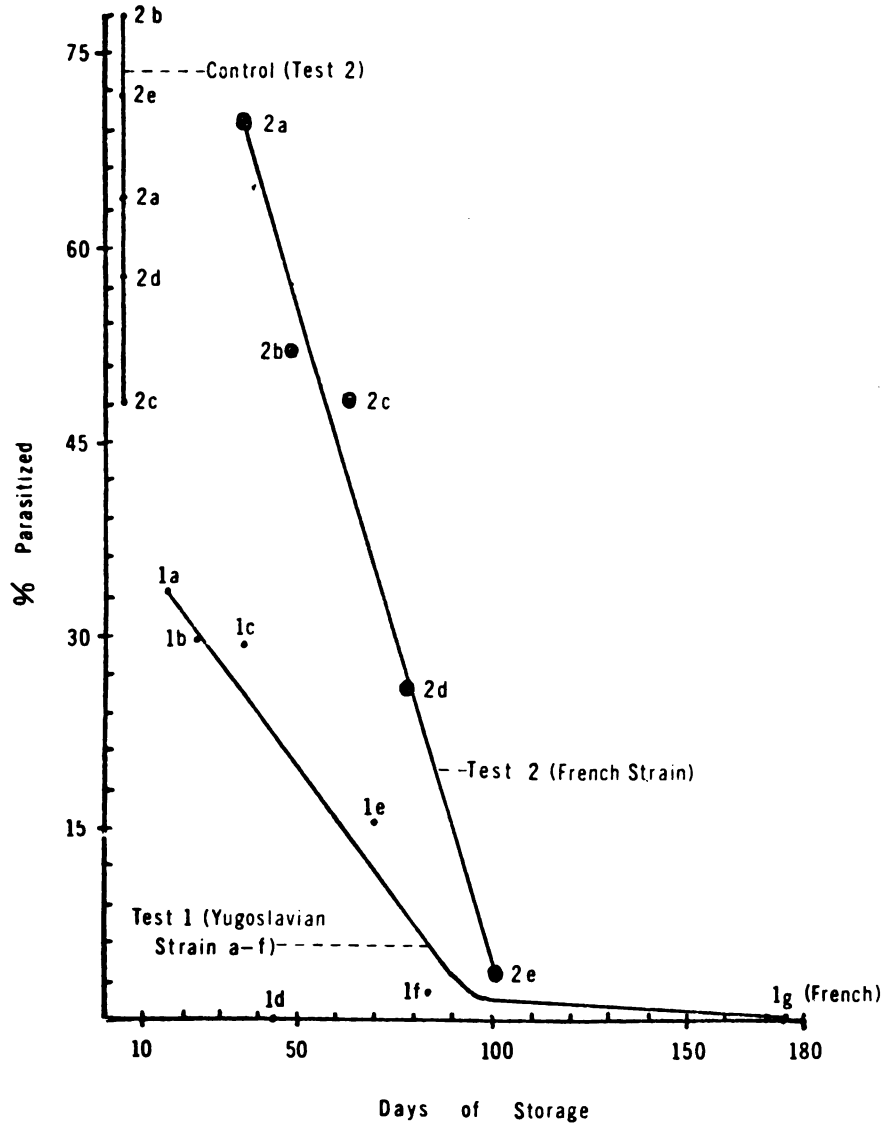


Figure 7 - Acceptability of CLB eggs to Anaphes when the eggs are stored over water up to 176 days.

slightly more fungus found on the eggs parasitized by the Yugoslavian strain, the mass culturing tests previously described indicate no differences between the French and Yugoslavian strains in terms of parasitized eggs per female used.

Use of Fungicides to Retard Growth of Fungus on Eggs During Storage

When old eggs are stored for long periods of time, fungus begins growing on them. It was believed that the fungal growth may be so great as to prevent parasites from ovipositing in the eggs, or if they are able to oviposit, then the offspring are unable to emerge. Consequently, various fungicides have been used in an attempt to eliminate the fungus. Among the fungi identified on CLB eggs were Aspergillus sp., Alternaria sp., and two species of Penicillium (Dr. Thanassoulopoulos, personal communication).

Captan, Semesan, Arasan, and Phygon have been used in the following storage tests. The use of these particular fungicides was suggested by Dr. Thanassoulopoulos.

The eggs, mounted in groups of 50 per 1/3 section of cut glass slide, were dipped in a bath of the given fungicide for 10 to 15 seconds and then rinsed twice in sterilized distilled water. Then, they were placed in 50 x 12 mm plastic disposable petri dishes (50 eggs per dish) and allowed to dry for 1 to 2 hours. Before storage, sterilized distilled water was placed in the dish to a level near that

of the top of the glass slide (none of the water came in contact with the eggs), the top of the dish was replaced, and the dish was stored at 40° F. The controls received no fungicide treatment but were dipped three times in sterilized distilled water except in Test 1 in which the controls were simply brought from storage and subjected to parasitism without being put through sterilized distilled water rinses.

Fungicide Test 1 - Captan.--The test solution contained 1 g of Captan per 100 ml of sterilized distilled water (5,000 ppm active ingredients). The eggs used were all from the same group of beetles, were a maximum of 2-days old, and were stored 1 day at 40° F without special treatment before being placed on slides. All slides had 50 eggs before they were placed in Captan, but during the dipping process a few eggs were washed from the slides; hence, dishes 1 to 8 did not have exactly 50 eggs.

When test eggs were taken from storage a control group of 50 eggs was used. Six female parasites per 50 eggs were used. After dish 3, fresh eggs were used for controls, since it was necessary to know whether failure in parasite development was due to treatment and/or storage or to inferior parasites. Table 9 gives the results of this test.

After 102 days, the number of eggs with black pupae in the test group is very much reduced compared to the controls, although three eggs were parasitized after 132 days

Table 9. The effect of Captan as a fungus inhibitor at the rate of 1 g (50% active ingredient) per 100 ml sterilized distilled water on cereal leaf beetle egg storage and subsequent parasitization by Anaphes flavipes

Dish No.	No. of Eggs		Days Eggs Stored		No. Eggs With Fungus[a]		No. BP[b]		Non-Emergence[c]		No. Adults			
	Test	Cont.[d]	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	Test ♀	Test ♂	Cont. ♀	Cont. ♂
1	49	50	18	17	0	28	41	42	0	1	55	29	70	19
2	47	50	29	19	1	44	20	25	3	5	29	8	34	11
3	49	54	48	48	0	49	17	14	0	8	24	10	13	1
4	47	41	83	7	4	36	7	4	1	0	7	1	4	2
5	48	50	102	2	9	46	12	16	2	6	15	2	20	4
6	47	50	120	1	0	37	0	32	0	7	0	0	46	7
7	49	49	132	23	0	49	3	18	0	0	3	0	35	6
8	45	53	177	5	0	17	0	25	0	0	0	0	40	14

[a]Fungus count was made 10 days after parasitization.

[b]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c]The number of parasitized eggs with no subsequent emergence.

[d]Cont. = untreated.

of storage. This test indicates that fungus can be effectively controlled on stored unparasitized CLB eggs with 1% Captan.

In the following tests, the eggs used were a maximum of 1-day old when removed from oviposition cages. They were stored for 4 to 11 days at 40° F before being used (during this time, they received no fungicide treatment). The controls were stored the same length of time and brought from storage with the test eggs. In addition, some test dishes had a second control composed of eggs stored no more than 9 days at 40° F that checked the effectiveness of the parasites. Five French female parasites were used in each dish of 50 eggs.

Fungicide Test 2 - Arasan.--For use in this test, .1 g Arasan 75 by Du Pont was mixed with 100 ml of sterilized distilled water (750 ppm active ingredients). Both the controls and test groups consisted of seven slides of 50 eggs each. The results of the test are in Table 10.

Dishes 5, 6, and 7 had a second set of controls used. When the results (see Table 10) of the tests and first controls are compared with the results of the second controls it is evident that the failure of the test and the first control eggs to develop more black pupae is not the result of weak parasites but instead is due to some other factor.

Table 10. The effect of Arasan as a fungus inhibitor at the rate of .1 g per 100 ml sterilized distilled water (750 ppm active ingredients) on cereal leaf beetle egg storage and subsequent parasitization by Anaphes flavipes

Dish No.	No. of Eggs		Days Eggs Stored		No. Eggs With Fungus[a]		No. BP[b]		Non-Emergence[c]		No. Adults			
	Test	Cont.[d]	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	Test ♀	Test ♂	Cont. ♀	Cont. ♂
1	49	47	16	16	5	25	31	26	2	0	61	7	50	9
2	50	49	29	29	2	29	6	28	0	0	6	3	61	8
3	50	50	43	43	13	50	19	28	1	0	20	15	42	10
4	50	47	58	58	4	35	5	8	0	0	8	2	17	4
5	50	49	73	73	1	48	1	5	0	0	3	0	10	3
2-5c[e]		53		5		20		24					40	14
6	50	50	89	89	16	30	0	6	0	0	0	0	10	3
2-6c[e]		50		9		30		30		0			53	10
7	50	50	98	98	0	49	0	0	0	0	0	0	0	0
2-7c[e]		50		3		22		41					89	16

[a]Fungus count was made 10 days after parasitization.

[b]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c]The number of parasitized eggs with no subsequent emergence.

[d]Cont. = untreated.

[e]The results are for the second controls.

Although .1 g Arasan has controlled the fungus effectively, parasitized egg development has been consistently less than for the controls except for dish 1.

Fungicide Test 3 - Phygon.--In this test, .1 g of Phygon per 100 ml of sterilized distilled water was used (500 ppm active ingredients). Both the test and control groups consisted of seven slides of 50 eggs each. The results of the test are presented in Table 11. The results indicate that .1 g of Phygon is not a very effective fungicide for this work in terms of fungus controlled.

Fungicide Test 4 - Semesan.--In the fourth test, .1 g of Semesan per 100 ml of sterilized distilled water was used (286 ppm active ingredients). The results are presented in Table 12.

The fungicide appears to have retarded fungal growth at least for the first 70 days of storage and at the same time allowed more eggs to develop black pupae and adults then the stored controls. But the test results are not good when dishes 4, 5, 6, and 7 are compared with the controls using fresh eggs.

Fungicide Test 5 - Captan.--In this test, .1 g of Captan per 100 ml of sterilized distilled water was used (500 ppm active ingredients). Ten slides were used with 50 eggs per slide for both the control and test groups. The results are presented in Table 13.

Table 11. The effect of Phygon as a fungus inhibitor at the rate of .1 g per 100 ml sterilized distilled water (500 ppm active ingredients) on cereal leaf beetle egg storage and subsequent parasitization by Anaphes flavipes

Dish No.	No. of Eggs		Days Eggs Stored		No. Eggs With Fungus[a]		No. BP[b]		Non-Emergence[a]		No. Adults Test ♀	No. Adults Test ♂	No. Adults Cont. ♀	No. Adults Cont. ♂
	Test	Cont.[d]	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.				
1	50	50	15	15	48	34	31	18	0	0	43	8	31	3
2	53	49	39	39	37	47	10	10	0	0	18	2	17	4
3	51	52	45	45	51	51	0	3	0	0	0	0	2	2
4	52	51	69	69	49	51	1	0	0	0	0	0	0	0
2- 4c[e]		53		5		20		24		0			40	14
5	50	50	85	85	45	50	0	0	0	0	0	0	0	0
2- 5c[e]		50		9		30		30		0			53	10
6	50	50	97	97	50	50	0	0	0	0	0	0	0	0
2- 6c[e]		50		6		7		21		0			30	9
7	50	52	119	119	50	50	0	0	0	0	0	0	0	0
2- 7c[e]		50		1		31		45		0			87	13

[a]Fungus count was made 10 days after parasitization.

[b]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c]The number of parasitized eggs with no subsequent emergence.

[d]Cont. - untreated.

[e]The results are for the second controls.

Table 12. The effect of Semesan as a fungus inhibitor at the rate of .1 g per 100 ml sterilized distilled water (286 ppm active ingredients) on cereal leaf beetle egg storage and subsequent parasitization by Anaphes flavipes

Dish No.	No. of Eggs		Days Eggs Stored		No. Eggs With Fungus[a]		No. BP[b]		Non-Emergence[c]		No. Adults Test		No. Adults Cont.	
	Test	Cont.[d]	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	♀	♂	♀	♂
1	51	49	15	15	14	25	35	20	0	2	74	13	40	5
2	49	50	39	39	20	50	25	11	0	1	30	27	14	4
3	50	50	45	45	31	49	4	6	0	3	5	3	5	0
4	50	50	69	69	28	49	11	5	0	1	18	7	5	5
2-4c[e]		53		5		20		24		0			40	14
5	53	50	85	85	53	50	5	0	0	0	10	1	0	0
2-5c[e]		50		9		30		30		0			53	10
6	50	50	97	97	46	50	0	0	0	0	0	0	0	0
2-6c[e]		50		6		7		21		0			30	9
7	51	52	119	119	35	50	0	0	0	0	0	0	0	0
2-7c[e]		50		1		31		45		0			87	13

[a] Fungus count was made 10 days after parasitization.

[b] BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c] The number of parasitized eggs with no subsequent emergence.

[d] Cont. = untreated.

[e] The results are for the second controls.

Table 13. The effect of Captan as a fungus inhibitor at the rate of .1 g per 100 ml sterilized distilled water (500 ppm active ingredients) on cereal leaf beetle egg storage and subsequent parasitization by Anaphes flavipes

Dish No.	No. of Eggs		Days Eggs Stored		No. Eggs With Fungus[a]		No. BP[b]		Non-Emergence[c]		No. Adults Test		No. Adults Cont.	
	Test	Cont.[d]	Test	Cont.	Test	Cont.	Test	Cont.	Test	Cont.	♀	♂	♀	♂
1	50	51	15	15	3	31	5	14	1	3	9	0	20	3
2	49	57	34	34	14	47	35	35	0	0	47	5	53	7
3	48	48	48	48	18	48	28	22	0	0	37	15	40	10
4	50	48	62	62	48	48	11	0	0	0	19	5	0	0
5	50	46	78	78	38	46	0	8	0	0	0	0	13	3
6	50	49	82	82	50	49	0	0	0	0	0	0	0	0
2-6c[f]		50		9		30		30		0			53	10
7	48	49	91	91	48	49	0	0	0	0	0	0	0	0
2-7c[f]		50		3		22		41		0			89	10
8	50	50	105	105	50	50	0	0	0	0	0	0	0	0
2-8c[f]		50		1		4		43		0			100	19
9	50	50	117	117	50	50	0	0	0	0	0	0	0	0
2-9c[f]		50		1		31		45		0			87	13
10	50	50	136	136	50[e]	50[e]								

[a]Fungus count was made 10 days after parasitization.

[b]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c]The number of parasitized eggs with no subsequent emergence.

[d]Cont. = untreated.

[e]This test was not run because all eggs were heavily covered with fungus and most were collapsed.

[f]The results are for the second controls.

This concentration of Captan is fairly good in retarding fungal growth and allowing satisfactory parasitism for the first 48 days of storage. After this time the fungal growth increases greatly and the number of eggs to develop black pupae drops off sharply.

To analyze the effectiveness of Phygon, Arasan, Semesan, and Captan (.1%) the test results were subjected twice to the two-way analysis of variance. In the first, the number of eggs to develop black pupae and the length of storage were analyzed; in the other, the number of eggs to develop fungus and the length of storage were analyzed. In both two-way analyses, the results of Arasan dish 5 were not used so that comparisons would be of equal size and of similar dates. In the other analysis, all dishes were assumed to have 50 eggs. The first Captan Test (1%) was not considered in the analysis primarily because more female parasites were used per 50 eggs and because the controls that were used were generally not in storage for the same period of time as the test eggs.

In terms of the development of parasites in the stored eggs, analysis revealed at the 5% and 1% levels of significance no difference between the tests and controls used. But in terms of fungal control, the analysis revealed that there is a highly significant difference (1% level) between Arasan and the other treatments. Thus, from the test results, it appears that at the rates tested Arasan is

the best of the fungicides in terms of fungus control. Analysis also revealed no difference between Semesan and .1% Captan in terms of fungus control; Phygon proved to be the worst of the fungicides tested.

Since the analysis indicated that none of the fungicide treatments decreased the development of parasites in unparasitized eggs held in storage at 40° F, and that fungus was effectively controlled at least by Arasan, .1% Captan, and Semesan, the failure to obtain larger numbers of parasites from stored unparasitized eggs as their length of storage increased was not the result of fungus but was due to some other factor or factors. Furthermore, the results of these tests were not caused by "poor" female parasites being used since in those cases where a second control of fresh eggs was used, much larger numbers of parasites developed than in the test eggs.

Freezing Eggs for Long Term Storage

Currently storage of unparasitized CLB eggs is conducted at 40° F. Possibly temperatures lower than 40° F might extend the CLB egg storage time.

Unparasitized eggs, mounted on glass slides within petri dishes with moist filter paper, were put into storage at approximately -12° F. Eggs were stored 2-1/2 and 14 hours, and 9, 20, 33, and 331 days. There were control eggs for only those eggs stored 20 and 33 days at -12° F. Table 14 presents the results of the test.

Table 14. The parasitization success of Anaphes flavipes on cereal leaf beetle eggs stored at -12° F for 2.5 and 14 hours, and 9, 20, 33, and 331 days

Time Eggs Stored	No. Eggs	No. F_1 Anaphes	
		Females	Males
2.5 hrs	25	41	11
14.0 hrs	25	42	12
9 days	20	0	0
20 days	20	0	0
33 days	20	0	0
331 days[a]	80	0	0
Unstored[b]	25	22[c]	

[a]Because 9 eggs collapsed during storage, only 71 eggs were actually subjected to parasitization.

[b]The unstored eggs were controls only for the eggs that were in storage 20 and 33 days.

[c]This is the total number of Anaphes flavipes that emerged.

The females actively parasitized the eggs stored 2.5 and 14 hours; they reacted as if the eggs were fresh. Anaphes appeared to normally "sting" the eggs stored 9, 20, 33, and 331 days but parasites failed to develop in the eggs. It appears that CLB eggs may be suitable for parasite development after a very short time, a matter of hours, at -12° F but that for any length of time in storage, this temperature is of no value since no parasite development was obtained from eggs stored for 9 days or longer at -12° F. CLB eggs freeze at approximately 7° F (Dickler, personal communication).

The Use of Extender for Egg Storage

Since direct freezing of eggs appears to show no promise for storing CLB eggs, the use of other materials to allow successful freezing of eggs has been tried. The basis of this storage has been the use of extender that is currently used in the storage of bull semen (H. D. Hafs, K. T. Kirton, C. Desjardins, unpublished report, 1962). This material is an egg-yolk-citrate extender containing glycerol. The extender usually consists of a portion without glycerol and an equal volume of the same extender that contains twice the amount of glycerol that is desired in the final mixture. The final mixture used here contains 7% glycerol.

Tests were conducted to discover whether Anaphes would develop in eggs treated with the extender. The unparasitized CLB eggs were immersed in extender and stored at 40° F. When brought from storage the eggs were either allowed to dry and then subjected to parasitism or they were given 1 to 2 minute rinses in physiological saline before Anaphes were placed with them. The tests indicated that Anaphes could develop from extender-treated eggs but that development was not satisfactory. In all instances the controls gave satisfactory parasite development. The test results are summarized in Table 15.

The tests indicated that a simple rinsing of eggs in physiological saline was not sufficient to allow satisfactory parasite development. Because of the results, treated eggs

Table 15. Parasitization of extender-treated cereal leaf beetle eggs that were stored at 40° F for various periods of time. The eggs were either dipped in physiological saline for approximately 1 to 2 minutes before parasitization or they were parasitized without the physiological saline treatment

Extender Treatment	Hours in Extender[a]	No. of Eggs	No. Anaphes Used	No. BP[b]	No. F_1 ♀	No. F_1 ♂	No. CLB Larvae to Develop and/or Emerge[c]	Saline Treatment
Non-glycerated	120	50	6	0	0	0	5	None
Non-glycerated	600	25	5	0	0	0	..[d]	1 rinse
7% glycerated	23	25	4	0	0	0	8	2 rinses
7% glycerated	37	49	3	0	0	0	26	3 rinses
7% glycerated	120	50	6	3	5	0	37	None
7% glycerated	600	50	10	0	0	0	..[d]	1 rinse
14% glycerated	120	50	6	2	1	0	32	None
14% glycerated	600	25	5	0	0	0	..[d]	1 rinse

[a]This is the same length of time the eggs were stored at 40° F while immersed in the extender.

[b]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c]CLB = cereal leaf beetle.

[d]The number of larvae to develop was not recorded.

were washed in the physiological saline for longer periods of time.

In the next test, eggs on glass slides were put in 7% and 14% glycerated extender and stored for 11 days at 40° F. When brought from storage, they were immersed in physiological saline for 5-3/4 hours, then placed on filter paper to dry before transfer to new glass slides for parasitization. Each group of 25 eggs had five female French Anaphes put on it. The controls consisted of 25, 1-day-old eggs that were in storage at 40° F for the same period as the test eggs. During storage, the controls had no moisture or extender added to them. All groups had some eggs develop parasites. The results are presented in Table 16.

Fifty 1-day-old eggs were covered with extender containing 7% glycerol and stored at 40° F for 16-1/2 hours; 50 untreated control eggs were also stored. When brought from storage, both the control and test eggs were immersed in physiological saline for 5 hours and then allowed to dry. Five female French Anaphes were used on each group of 25 eggs. From the test eggs five CLB larvae hatched and 31 developed black pupae producing 73 parasites. From the controls, 42 eggs with black pupae developed yielding 83 parasites.

Test results indicate satisfactory parasite development in eggs treated with glycerated extender provided they are washed in physiological saline for 5 hours (although a

Table 16. Parasitization success of Anaphes flavipes on cereal leaf beetle eggs stored with non-glycerated and 7% and 14% glycerated extender for 11 days, with a 5-3/4 hour rinse of physiological saline before parasitization

Test	No. Eggs	No. Used	No. BP[a]	F_1 ♀	F_1 ♂
Without glycerol	25	5	7	9	1
With glycerol[b]	25	5	22	31	28
With both portions[c]	50	10	24	29	26
Untreated	25	5	20	26	32

[a]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[b]Only the glycerated portion of the extender (14% glycerol).

[c]With equal volumes of the glycerated and non-glycerated portion of the extender (7% glycerol).

shorter time interval might be satisfactory) and then dried before parasitization.

The extender tests were conducted to discover whether Anaphes would develop from the treated eggs so that if they did develop, storage of unparasitized eggs could be attempted using the method for storage of bull semen which with the use of extender (7% glycerated) is stored in liquid nitrogen (-196° C). Since Anaphes can develop from extender-treated eggs, a number of eggs were treated with extender and stored in liquid nitrogen.

Into each of ten, 1 ml vials used for storing bull semen were placed 25, 1-day-old CLB eggs (stored at 40° F

1 day before use). In five vials, the eggs were left on pieces of the plant leaves and in the other five, three to four eggs were mounted on the inside wall of each vial with the remaining eggs mounted on those attached to the vial. The vials, when filled with 7% glycerated extender, were heat sealed and allowed to equilibrate for approximately 20 hours. The vials were then stored in liquid nitrogen by Charles Cornell of the Michigan Animal Breeder's Cooperative utilizing their method for bull semen storage.

Five days after storage, the five vials containing eggs mounted on leaves were removed from storage. The vials, left at room temperature for approximately 3 hours, were opened and the eggs immersed in physiological saline from 5-1/2 to 6-3/4 hours. Table 17 presents the results of the test.

All the eggs were dried 2-1/2 to 3 hours after removal from saline and then the eggs were removed from the leaves and placed on glass slides. All eggs used looked fresh and ovipositor insertion was observed in two eggs of dish 4.

The 25, 1-day-old control eggs had been stored 2 days at 40° F before use. These eggs were immersed in physiological saline for approximately 3 hours and after drying, five Anaphes were placed on the eggs. Nothing developed from the test eggs although 14 control eggs were parasitized yielding 33 female and 5 male Anaphes. In

Table 17. The effects on cereal leaf beetle eggs and parasitization success of Anaphes flavipes on the eggs after storage for 5 days in liquid nitrogen

Dish No.	No. Eggs	No. Eggs to Collapse in Storage	No. Eggs Broken in Handling	No. Eggs Used	No. ♀ Used	Hours in Saline	No. Anaphes and/or Beetle Larvae to Develop
1	25	4	8	13	3	5-1/2	0
2	25	4	7	14	3	5-3/4	0
3	25	5	10	10	2	6	0
4	25	4	5	16	3	6-1/2[a]	0
5	25	3	6	16	3	6-3/4[b]	0
6[c]	25	..	..	25	5	3	38[d]

[a]These eggs were given two consecutive treatments of saline, the first for 6 hours and the other for 1/2 hour.

[b]These eggs were given two consecutive treatments of saline, the first for 6 hours and the other for 3/4 hour.

[c]These eggs were the controls.

[d]These were parasites; 5 CLB larvae also hatched.

addition, five CLB larvae hatched from the controls and none hatched or developed from the test eggs.

The remaining five groups of eggs were removed from liquid nitrogen 17 days later. All the eggs, left at room temperature for 1 day after storage, were then placed in physiological saline from 5-3/4 hours to 72 hours. The eggs were dried 15 minutes on filter paper and then placed on glass slides; after the eggs were completely dried, parasites were placed with the eggs. The eggs not used in each dish were either destroyed in storage or in handling after storage. Table 18 lists the number of eggs used and the physiological saline treatment time for each set of eggs. Upon the 50 2-day-old unstored control eggs were placed five Anaphes.

Dish 4 was dropped and the eggs destroyed. When checked 72 hours after immersion in physiological saline, all eggs of dish 5 were found to have collapsed. Nothing developed from the test eggs whereas 27 control eggs were parasitized and from which 22 female and 38 male Anaphes emerged.

Although Anaphes develops from eggs treated with glycerated extender and held at 40° F, they do not appear to develop from eggs treated with 7% glycerated extender and then stored at -196° C under the conditions used here. In addition, the eggs appear to be more fragile after nitrogen storage than do those that have not been in nitrogen storage.

Table 18. Parasitization success of Anaphes flavipes on cereal leaf beetle eggs after storage for 17 days in liquid nitrogen

Dish No.	No. Eggs	No. Eggs Used[a]	No. ♀ Anaphes Used	Hours in Saline	No. Anaphes and/or Beetle Larvae to Develop
1	25	7	2	5-3/4	0
2	25	12	2	5-3/4	0
3	25	10	2	5-3/4	0
4	25	9	..[b]	...	..
5	25	0	..	72	..
6[c]	50	50	5	0	60[d]

[a]The balance of the eggs were either damaged during storage or in handling.

[b]The dish was dropped before the eggs could be dried.

[c]These are the control eggs and were not stored in liquid nitrogen.

[d]These were all Anaphes; no CLB larvae developed.

Gas Atmosphere Storage of Eggs

As early as 1821, M. Barard reported that storage in oxygen-free atmospheres could be used to preserve fresh fruits (Harvey, 1967). But according to Harvey (1967) it was not until almost 100 years later that work by Kidd and West in 1920 provided a base for the commercial development of controlled atmosphere storage.

In 1940 the first commercial trials involving controlled atmosphere storage were begun in North America (Porritt, 1963). For the United States, controlled

atmosphere storage has lengthened storage life of certain apple varieties. In addition, a number of problems such as soft scald and brown core have been almost eliminated with the use of controlled atmosphere storage (Van Doren, 1961). Harvey (1967) states that as of 1965, 15 million bushels of apples were in controlled atmosphere storage in the United States. In addition to apples, controlled atmosphere tests have been conducted on strawberries, peaches, sweet cherries, lettuce, tomatoes, pears, and various other fruits and vegetables.

Most controlled atmospheres are obtained by allowing the stored commodity to modify the atmosphere through respiration in a sealed room or container. When the desired level of oxygen is reached, enough outside air is admitted to maintain the oxygen at that level. When the carbon dioxide reaches the desired level, the excess carbon dioxide is removed by passing the atmosphere through some material such as caustic soda that will absorb carbon dioxide. Both processes are carefully controlled to provide the proportion of oxygen and carbon dioxide that best maintain quality for the particular variety of fruit being stored (Harvey, 1967).

Low-oxygen atmospheres, by slowing the respiration rates of fruits and vegetables, lengthen their storage or market life. High carbon dioxide levels, like low-oxygen levels, slow the respiration rate and are even more effective than low oxygen in retarding decay.

Controlled atmosphere storage is a supplement to refrigeration but not a substitute since temperature is a controlling factor in determining the effects of controlled atmospheres used in storage. Fruits and vegetables such as apples and pears that are harvested green and ripen after harvest are most affected by controlled atmosphere storage whereas fruits such as grapes that do not ripen further after harvest do not respond significantly to controlled atmosphere.

In addition each variety of fruit or vegetable responds differently to a particular atmosphere. Thus, in mixed loads no single combination of gases would provide optimum conditions for all commodities being stored.

Because of the success in controlled atmosphere storage of apples and other fruit and vegetables, gas atmosphere were used in an attempt to develop a more satisfactory means of storing unparasitized CLB eggs. Storage was conducted at 40^{o} F and lower temperatures using 100% carbon dioxide (CO_2), 100% nitrogen (N_2), a mixture of 5% carbon dioxide and 95% oxygen (CO_2/O_2), and a mixture of 12% carbon dioxide, 79% nitrogen, and 9% oxygen ($CO_2/N_2/O_2$).

The first tests were pilot tests to see if Anaphes could develop from eggs stored in various gas atmospheres. In the test 25, 2-day-old eggs, were mounted on strips of plastic large enough to fit into either 25 or 50 ml Erlenmeyer flasks. The flask contained a piece of sterilized

cotton which was saturated with sterilized distilled water. After the plastic strip with the eggs was placed in the flask, it was flushed with the appropriate gas for 2 to 3 minutes before the top was sealed with a sleeve-type rubber stopper (see Fig. 8). The flasks were then placed in storage at 40° F. The results are presented in Table 19.

Table 19. Parasitization success of Anaphes flavipes on unparasitized cereal leaf beetle eggs stored for 11 days at 40° F in gas atmospheres of air, 100% nitrogen (N_2), 100% carbon dioxide (CO_2), and mixtures of 5% carbon dioxide, 95% oxygen (CO_2/O_2), and 12% carbon dioxide, 79% nitrogen, and 9% oxygen ($CO_2/N_2/O_2$)(25 eggs per flask)

Flask No.	Gas	No. BP[a]	No. Offspring ♀	No. Offspring ♂	No. Eggs with Fungus After Storage[b]	No. Eggs with Fungus 9 Days After Storage
1	Air	15	33	4	6	10
2	Air	22	43	6	1	7
1	N_2	18	32	5	0	16
2	N_2	15	30	2	1	14
1	CO_2	23	39	7	2	3
2	CO_2	20	29	2	5	7
1	CO_2/O_2	6	13	1	0	5
2	CO_2/O_2	19	40	7	1	10
1	$CO_2/N_2/O_2$	21	38	6	0	16
2	$CO_2/N_2/O_2$	14	19	3	2	22

[a]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[b]The fungus count was made at the time that the eggs were removed from storage.

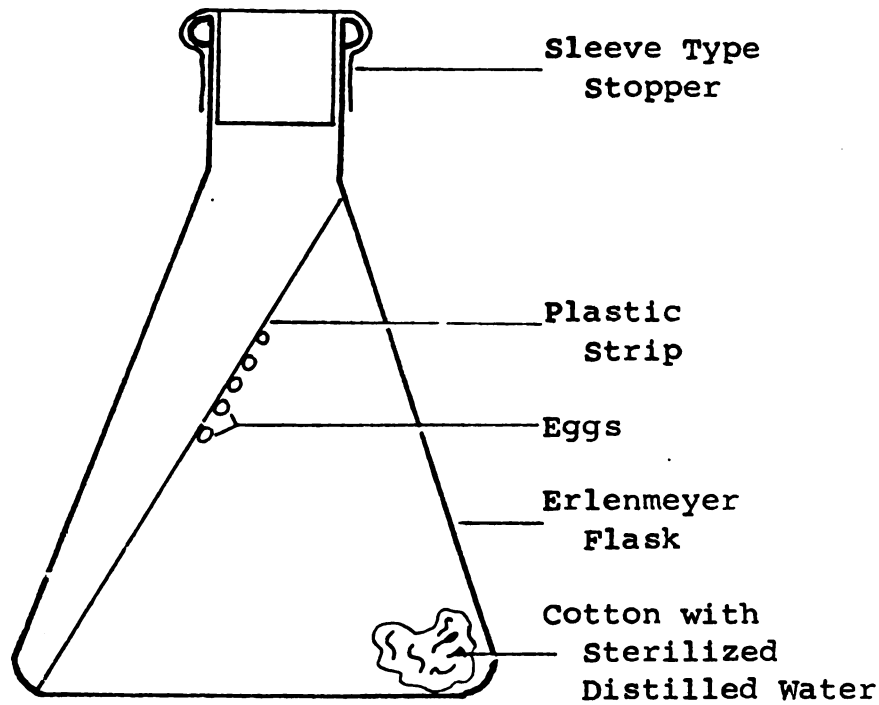


Figure 8 - The container used for storing CLB eggs in various gas atmospheres.

All the gases used except for $CO_2/N_2/O_2$ came from large metal cylinders used for storing gases. The exception was in an inner tube fitted with a special valve. With $CO_2/N_2/O_2$, the flasks were flushed for 1 minute although the amount of gas actually in the flasks may be questionable due to the inner-tube apparatus used. The eggs exposed to N_2 and CO_2/O_2 were in 50 ml flasks while the remaining eggs were in 25 ml flasks.

The results of the test indicated that for the first 11 days of storage at 40° F using the various gas atmospheres, parasites developed quite well from the stored eggs. Since development did occur in the eggs, it was decided to conduct a series of tests that involved egg storage in the gas atmospheres for longer periods of time.

In this series of tests a total of 2000 eggs were stored, 500 eggs to be brought out at monthly intervals. One-half of them were stored at 40° F and the other half at 14° F. The eggs, a maximum of 1-day-old, were mounted in groups of 25 per strip of clear plastic, approximately 50 x 10 mm. Half of the eggs stored at each temperature were treated with Captan (.1 g/100 ml sterilized distilled water) before subjecting them to the gases. The Captan treatment consisted of dipping the eggs into Captan for about 15 seconds and then dipping them into two separate rinses of sterilized distilled water. After drying, the eggs were placed in 25 ml Erlenmeyer flasks with a small piece of

sterilized cotton that had been saturated with sterilized distilled water. Within 24 hours from the time the eggs were mounted, the flasks were flushed with the appropriate gas, stoppered, and stored at 40° F.

The flasks to be stored at 14° F were first stored for 6 days at 40° F. When stored at 14° F, they were placed within a styrofoam box which helped to maintain a constant temperature around the eggs. Beginning with Test 3 (stored approximately 100 days) a set of controls consisting of fresh eggs were used. The control eggs were used to test the Anaphes for their ability to produce offspring. These eggs were in 40° F air storage for no more than 12 days. The test results for eggs stored at 40° F are presented in Table 20.

This set of tests indicate that for the first 30 days, eggs may be stored at 40° F in the various gas atmospheres tested and satisfactory parasite development can be obtained. The only exception to the gases used would be 100% CO_2 which had about half the number of parasitized eggs develop as did the other gases or air after 30 days of storage. Figure 9 presents graphically both the results of the pilot test and the succeeding gas atmosphere test. The slight differences in terms of parasitized eggs between the pilot test (11 days) and the test results after 30 days are probably due to the fact that these were different tests beginning at different times and using both eggs and parasites from different groups.

Table 20. Parasitization success of Anaphes flavipes on unparasitized cereal leaf beetle eggs stored for various time periods at 40° F in gas atmospheres of air, 100% nitrogen (N_2), 100% carbon dioxide (CO_2), and mixtures of 5% carbon dioxide and 95% oxygen (CO_2/O_2), and 12% carbon dioxide, 79% nitrogen, and 9% oxygen ($CO_2/N_2/O_2$)

Test No.	Gas	No. Eggs	Days Eggs Stored	Captan	No. BP[a]	No. Anaphes ♀	No. Anaphes ♂	No. Eggs With Fungus[b]	No. of CLB Larvae[c]
1	Air	25	33	Yes	23	46	8	11	0
	Air	25	33	No	14	20	4	4	8
	N_2	25	33	Yes	22	31	4	3	0
	N_2	25	33	No	15	23	5	19	2
	CO_2	25	31	Yes	8	11	2	0	0
	CO_2	25	31	No	7	8	1	21	0
	CO_2/O_2	25	33	Yes	23	31	6	1	0
	CO_2/O_2	25	33	No	10	19	3	6	2
	$CO_2/N_2/O_2$	25	32	Yes	20	25	10	0	0
	$CO_2/N_2/O_2$	25	32	No	18	7	21	16	1
2	Air	25	67	Yes	9	14	1	16	0
	Air	25	66	No	4	0	0	0	0
	N_2	25	67	Yes	6	7	1	0	0
	N_2	25	66	No	0	0	0	0	0
	CO_2	25	65	Yes	0	0	0	4	0
	CO_2	25	64	No	1	2	0	25	0
	CO_2/O_2	25	67	Yes	0	0	0	0	0
	CO_2/O_2	25	66	No	0	0	0	25	0
	$CO_2/N_2/O_2$	25	66	Yes	8	5	2	23	0
	$CO_2/N_2/O_2$	25	65	No	0	0	0	25	0
3	Air	25	105	Yes	0	0	0	12	0
	Air	25	101	No	..[d]	..	..	..	..
	N_2	25	105	Yes	0	0	0	0	0
	N_2	25	101	No	0	0	0	25	0
	CO_2	25	103	Yes	1	0	1	0	1
	CO_2	25	99	No	0	0	0	25	0
	CO_2/O_2	25	105	Yes	0	0	0	6	0
	CO_2/O_2	25	101	No	1[e]	..	..	25	0
	$CO_2/N_2/O_2$	25	104	Yes	0	0	0	0	0
	$CO_2/N_2/O_2$	25	100	No	1	1	0	25	0
Control 1[f]		50			24	0	53		
Control 2[g]		25			20	38	5		
4	Air	25	123	Yes	..[d]	..	..	..	..
	Air	25	122	No	0[d]	0	0	25	0
	N_2	25	123	Yes	..	..	..	..	..
	N_2	25	122	No	0	0	0	25	0
	CO_2	25	121	Yes	0	0	0	0	0
	CO_2	25	120	No	0	0	0	25	0
	CO_2/O_2	25	123	Yes	0[d]	0	0	0	0
	CO_2/O_2	25	122	No	..	..	..	..	..
	$CO_2/N_2/O_2$	25	122	Yes	0	0	0	0	0
	$CO_2/N_2/O_2$	25	121	No	2	1	0	25	0
Control 1[f]		25			21	29	23		
Control 2[g]		25			9	9	3		

[a] BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[b] The fungus count was made 9 days after the eggs were brought from storage.

[c] The number of cereal leaf beetle larvae to develop (none hatched).

[d] The eggs were too heavily covered with fungus so Anaphes was not placed on the eggs.

[e] No Anaphes emerged from the one parasitized egg.

[f] Fresh egg (untreated) controls for the test eggs treated with Captan.

[g] Fresh egg (untreated) controls for the test eggs not treated with Captan.

The results indicated that after approximately 65 days of storage at 40° F some eggs from several of the gases tested still developed black pupae but the numbers were not as great as for the controls (air) and the controls did not do well (only 13 of 50 eggs developed black pupae). (See Fig. 9). Even after approximately 100 days storage at 40° F one parasitized egg developed from those eggs stored in each gas atmosphere containing CO_2 and 2 parasitized eggs were obtained from eggs stored 121 days in $CO_2/N_2/O_2$. At 14° F storage, the tests revealed that no gas mixture (including air) resulted in parasitized eggs (there was one exception: one egg stored in air for 33 days supported the development of parasites after storage).

The testing further revealed that with this method of storage at either 40° F or 14° F fungicide treatment is probably necessary for fungal control with the various gas mixtures tested. Almost all the Captan-treated eggs stored at both 14° F and 40° F had much less fungus than did the eggs without Captan. Furthermore, treated eggs usually yielded more parasitized eggs than did the untreated eggs.

The yield in parasitized eggs from those parasitized after storage at 40° F in gas atmospheres is no greater than from those eggs stored in air. The ability of eggs stored in gas atmospheres to support development of parasites as storage time increases is no greater than for eggs stored in air. Therefore the gas atmospheres tested offer no better

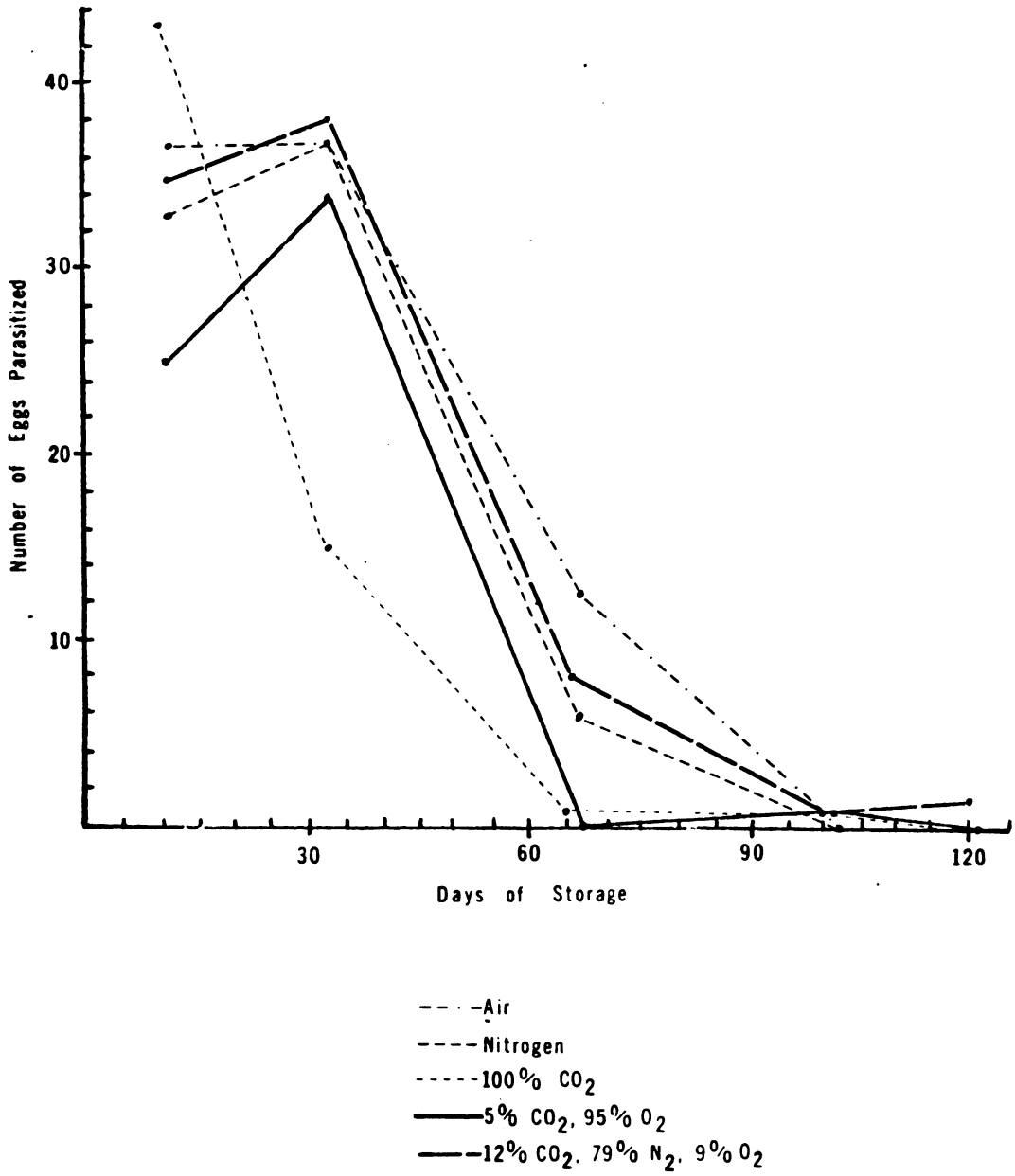


Figure 9 - Storage of unparasitized CLB eggs at 40° F in various gas atmospheres.

means of storage than is presently used. But it is possible that other gases or proportions and/or temperatures might perform better than those used here. At 14° F, storage is definitely not desirable since almost no parasitized eggs developed after 30 days of storage. Assuming the eggs stored at 14° F had satisfactory development before 30 days of storage, this would be of no benefit since the current technique of storage at 40° F gives satisfactory development for 30 days and longer. Furthermore, the current storage technique is less complicated than the use of the gas atmospheres.

STORAGE OF PARASITIZED EGGS

Occasionally it is necessary to store parasitized eggs due to such factors as insufficient CLB eggs or parasitization. Currently the only practical way to store Anaphes is as black pupae. This stage occurs within the CLB egg from 1 to 2 days before the adult parasite emerges. At this time the egg takes on the dark color of the adult parasite.

CLB eggs containing black pupae were held in storage at 40° F for 7 weeks. The parasitized eggs, mounted on glass slides, were placed in petri dishes that contained filter paper to which sterilized distilled water was added every other day. Each week enough parasitized eggs were removed from storage to provide a minimum of 15 female Anaphes plus males for mating. The most vigorous individuals were used in the test (see Table 21). The females were placed with 100 CLB eggs.

The results indicate that it is possible to hold pupae as long as 7 weeks at 40° F without completely eliminating mating or viable sperm. But since there were no controls, it is not possible to say whether the unusually high proportion of male F_1 is due to the storage of the parents as black pupae or due to insufficient mating time allowed the parasites after emergence.

Table 21. Survival and parasitization success of parasites emerging from Anaphes flavipes pupae stored at 40° F for 1-7 weeks

Weeks Stored	No. Adults to Emerge After Storage ♀	♂	No. Adults Used ♀	♂	No. Eggs Used	No. F_1 ♀	♂	Total F_1
1	17	9	15	9	100	36	70	106
2	23	10	15	7[a]	100	33	41	74
3	35	11	15	7[a]	100	53	40	93
4	27	12	15	6[a]	100	0	29	29
5	28	8	15	7[a]	100	28	49	77
6	31	7	15	5[a]	100	0	59	59
7	14	15	8[a]	8[a]	100	35	23	58

[a]Fewer were used than emerged because some were weak, dying, or dead.

The results indicate that with an increase in the storage time of the parents as black pupae there is a general decline in the number of F_1 to develop, although the last test seems to negate this general trend since only eight females (instead of 15) were available. It is possible that all eight females used in the last test were strong and the weak ones had died before any could be placed on the eggs. In the other tests, the most active and vigorous females were chosen, but it is possible that some of these were not as strong as they seemed and oviposited little or not at all. This would mean that, even though 15 females were present, only part of them were actually ovipositing.

Adults emerging from pupae held up to 2 weeks in storage at 40° F appear to be as active as adults emerging from a continuous culture. Beyond 2 weeks there is some reduction in vigor and activity and beyond 4 weeks the reduction is quite noticeable, resulting in specimens whose activity is considerably less than adults emerging from unstored pupae. The loss of vigor and activity of adult parasites as the length of black pupal storage increases has been noted by workers at the Niles Lab (Moorehead, personal communication).

Storage of Parasitized Eggs Earlier than the Black Pupal Stage

The general procedure used in storage of parasitized eggs consists of placing the eggs in petri dishes with moist filter paper and then storing at 40° F. Fungus is a problem in the storage and is partly aleviated by the use of fungicide on the unparasitized eggs since protection carries over to the eggs after parasitization.

In an attempt to find a better means of storing parasitized eggs, it was thought that storing eggs in life stages earlier than the black pupal stage might be useful. Thus, eggs were stored at 40° F 2 days, 4 days, 6 days, and 8 days after adult parasites were put on the eggs.

The parasitized eggs were stored in the following manner: the eggs, five rows of 10 eggs each per 1/3 section of cut glass slide were left in the petri dish (50 x 12 mm

disposable plastic petri dishes with tight fitting lids) in which they were stung. Filter paper was placed in the bottom of the petri dishes and then sterilized distilled water was placed in the dish to a level nearly equal to the height of the glass slide (the eggs were not submerged in the water). The dishes were then put into storage.

In all but the first three tests, five females were used per 50 eggs. In the first three tests, six females were used. The females were confined with males in gelatin capsules for approximately 1 hour for mating, then watered and fed for 2 to 4 hours before being used.

The results of the first three tests are not given because nothing emerged after the first set of parasitized eggs were brought from storage. The lack of emergence was apparently the result of fungus.

After the first three tests were concluded, it became clear that a fungicide was necessary during storage to retard fungal growth. Thus, the eggs in the next test were treated with Captan 3 days after the parasites were placed on the eggs (1 g of Captan per 100 ml of sterilized distilled water or 5000 ppm active ingredients). Treatment consisted of the eggs being dipped into the Captan solution and then into two rinses of sterilized distilled water. Eggs stored 2 days after being parasitized were treated with Captan an hour before they were stored. The results are presented in Table 22.

Table 22. Survival and emergence of Anaphes flavipes after storage of parasitized cereal leaf beetle eggs with various aged developmental stages of the parasite (50 eggs per dish)

Dish	No. Days Eggs Exposed to Parasites Before Storage	No. Days Eggs Stored	No. BP[a]	No. of Offspring ♀	No. of Offspring ♂	No. of Parasitized Eggs with No Emergence
1	2	15	15	14	4	3
2	2	29	20	15	10	4
3	2	43	28	26	5	9
4	2	57	32	10	4	22
5	4	17	11	12	2	0
6	4	33	2	2	0	0
7	4	43	13	7	2	4
8	4	57	15	0	0	15
9	6	15	22	17	13	3
10	6	31	23	14	2	13
11	6	43	37	2	0	36
12	6	57	31	0	0	31
13	8	15	10	..[b]	..	..
14	8	29	36	46	10	6
15	8	43	37	7	5	31
16	8	57	38	4	6	31
Control 1		0	35	40	23	0
Control 2		0	45	86	14	3

[a]BP = the number of parasitized CLB eggs to develop black pupae.

[b]The eggs dried out before the parasites could emerge.

The number of parasitized eggs with no parasite emergence in dishes 1, 2, 5, 6, 9, 10, 13, and 14 was primarily due to the collapse of the eggs by the time the remaining parasitized eggs had parasite emergence. In dish 4, the eggs with black pupae that failed to complete development looked good, but for some reason adults did not emerge.

In dish 7, the parasites that emerged appeared to be normal. In dish 8, none of the parasitized eggs had emergence although one egg had a female Anaphes that was alive--she could be seen moving within the egg--but she failed to emerge.

From dish 10, one of the females to emerge had malformed wings and antennae. Although no males emerged with her, she was placed with 14 eggs that were a maximum of 1-day old and had been stored 5 days before use. The parasite did not appear to be interested in the eggs. Cereal leaf beetle larvae hatched from eight of the eggs and the other six collapsed with nothing developing.

In dish 11, the eggs developed black pupae from 4 July to 6 July, 1967. As of 12 July only one of the eggs with black pupae had had adults emerge. There appeared to be no movement in the other eggs containing black pupae. Since it was unknown whether these black pupae were dead or in diapause, they were replaced in storage in an attempt to break diapause if this were the cause. At this time,

21 eggs with black pupae still looked good. The other eggs with black pupae were either covered with fungus or had collapsed. The eggs in dish 11 were periodically checked but after 3 months all the eggs had collapsed and had fungus covering them.

In dish 12, the eggs with black pupae looked good but parasites failed to emerge. When dish 13 was brought from storage the top of the dish was accidentally left off and the eggs dried out before the adults could emerge. In dish 15 and 16 all the parasites that emerged had deformed wings.

Figure 10 shows the results of the storage tests. As in the case with the storage of unparasitized eggs, there appears to be a definite period of storage after which parasite survival begins to decline rapidly; the time seems to be between 30 and 40 days.

In addition, it is evident (Table 22) that as the storage time increases, there is also an increase in the number of parasites that do not emerge. The decreased emergence may be due to parasites going into diapause, but this has not been shown. The eggs with unemerged black pupae collapsed, both when left at room temperature and when replaced in storage and checked periodically.

By the use of a two-way analysis of variance, at the 1% level of significance there is no difference between eggs stored 2 days after introduction of parasites and 6 days

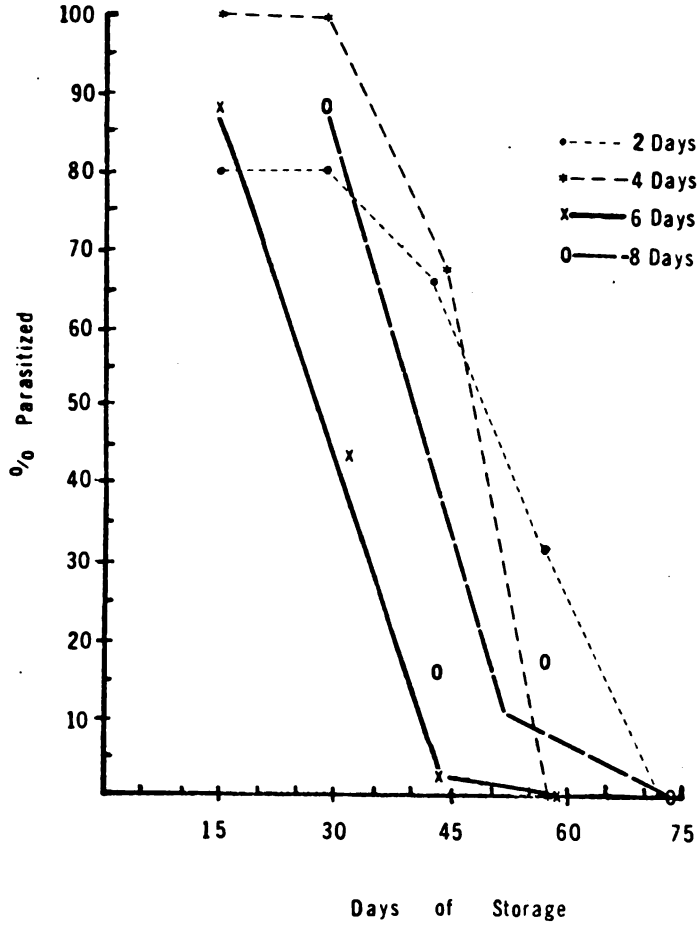


Figure 10 - The results of tests to determine the optimum age of Anaphes flavipes for storage at 40° F. The eggs were stored 2, 4, 6, and 8 days after parasites were placed with the eggs.

after introduction and also between 6 days and 8 days after parasite introduction. Eggs stored 4 days after the introduction of the parasites had the fewest parasitized eggs develop and in terms of the number of parasitized eggs was significantly different from the eggs stored 2, 6, and 8 days after the introduction of parasites. Egg storage 2 and 4 days after parasite introduction is not as good as storage 6 or 8 days after parasite introduction. Since the eggs stored at 8 days after parasite introduction are close to or are black pupae, this group of stored eggs is similar to those that are regularly stored.

COLD STORAGE OF ADULT PARASITES

As an alternative to the storage of black pupae, an attempt was made at storing adult parasites. Into a petri dish containing moist filter paper were placed 12 female and 5 male French Anaphes. The top of the dish was taped to the bottom and then stored at 40° F. At the same time 10 female parasites that had previously parasitized eggs were stored in another petri dish under the same conditions. The adults were fed and watered each time the dishes were checked for live parasites. Tables 23 and 24 give the results of the tests.

Table 23. Survival of 17 female and male Anaphes flavipes (French strain) stored at 40° F

Days Stored	No. Live Parasites	
	♀	♂
0	12	5
2	11	4
4	11	4
6	9	2
8	..[a]	..

[a]The dish was dropped.

Table 24. Survival of female Anaphes flavipes (French strain) stored at 40° F

Days Stored	No. Live Parasites
0	10
2	9
4	8[a]
6	8
8	4
11	2
13	1
15	1
17	0

[a]One parasite was lost while being transferred to a dish with honey and moisture.

The same conditions were used in the next test. A petri dish with 10 males was stored at 40° F. The results are in Table 25.

Table 25. Survival of 10 male Anaphes flavipes (French strain) stored at 40° F

Days Stored	No. Live Parasites
0	10
2	7
4	5
7	1
9	0

Three dishes with parasites were used in another test. One dish with 9 adult males and moist filter paper was left at room temperature and used as control. The second dish, stored at 40° F, contained moist filter paper

and 8 males. Nine males were stored at 40° F in the third dish which contained moist filter paper with honey. The results of this test are in Table 26.

Table 26. Survival of adult male Anaphes flavipes (French strain) at room temperature and stored with and without honey at 40° F

	Number of Live Parasites		
Days Stored	Room Temperature	Without Honey	With Honey
0	9	8	9
2	0	3	9
4	0	2	3
6	0	0	0

At 40° F in the laboratory about 50% female mortality occurs after 1 week of storage and 50% male mortality about 3 to 4 days after commencement of storage. It appears as a result of these tests that adult Anaphes cannot be effectively stored for long periods of time under the conditions used. In those instances where parasites survived for a period of time in storage, they were much less active when brought out than are newly emerged adults. Adult Anaphes storage is not an effective means of long term storage.

Laboratory cultures are not exposed to the environment the way field populations are. Whether or not field conditioning would enable the parasites to survive the summer and winter as adults after CLB eggs are gone is unknown.

X-RAY TREATMENTS OF CLB ADULTS AND EGGS

X-ray treatments were given CLB eggs to determine whether CLB larval development could be terminated and at the same time obtain parasite development. If parasite development could be obtained, then in the field release of parasite pupae there would be no addition of CLB larvae to the CLB population already present in the field.

Adult Beetle Treatment

According to Brennan (1967) female CLBs are more sensitive to radiation than males. When females are treated with X-ray dosages of 2000 roentgens or greater, egg production is almost eliminated. In addition, Brennan states that when untreated females are mated to males that have been treated with 2000 rads (93 roentgens = 100 rads) of beta radiation about 87% of the eggs laid are infertile. Untreated females mated to adult males treated at 1000 rads results in about 50% egg viability whereas at the 4000 rad level viability is almost reduced to zero (W. Myser, unpublished report, 1967). Egg production is not adversely affected by mating unirradiated females to males irradiated up to 8,000 rads (W. Myser, unpublished report, 1967).

Above 2000 roentgens, according to Brennen (1967) mortality of pre-diapause males begins to increase greatly. At the 2000 roentgens level mortality is much greater for the first 15 days after treatment of post-diapause beetles than for untreated males.

Because irradiation of females appears to affect oviposition, it was decided to treat males with 2000 roentgens of X-ray. In the test, all the beetles used were reared in the laboratory and in diapause storage for approximately 12 weeks before use. They were sexed, using the technique developed by Myser and Schultz (1967), as soon as they were removed from storage. Since females do not begin oocyte development until after the cessation of diapause (Hoopingarner, Kumararaj, and French, 1965) those used in the test were virgins.

The males while confined in a plastic disposable petri dish were treated with a Maximar 250-III machine for 6.45 minutes at 310 r/min. The 50 treated males were divided into two groups of 25 each; each group was placed with 17 untreated females from the same storage container from which the males and controls came. All the eggs to be used in the testing were subjected to parasitism at room temperature. Fifty untreated and unsexed adults were used as controls.

Test 1.--In this test, there were 109 eggs from the treated beetles and 37 eggs from the controls. The eggs were a maximum of 3-days old and stored for 5 days at 40° F before they were used.

In the test group two petri dishes each had 50 eggs and a third dish had nine eggs. One petri dish contained control eggs. Filter paper was placed in the bottom of the dishes and kept moist with sterilized distilled water during the test.

In the test group, five females were placed in each dish with 50 eggs and two were placed with the nine eggs. Five French Anaphes were used on the controls. The results are listed in Table 27.

Test 2.--In this test, there were 110 test eggs and 30 control eggs, a maximum of 2-days old. The eggs, without fungicide treatment, were stored for 3 days at 40° F before use.

Two petri dishes contained 50 test eggs each and one contained 10 eggs. Five female French Anaphes were used in each dish of 50 eggs and two females were put in the dish with 10 eggs. Five French female Anaphes were used on the controls. Table 27 presents the results of this test.

It is evident that parasites do develop from eggs laid by unirradiated female CLBs that have been mated with males treated with 2000 roentgens X-ray, but there may be some difference in the parasitization. The control eggs

Table 27. Parasitization success of Anaphes flavipes in eggs from untreated female cereal leaf beetles mated with male cereal leaf beetles exposed to 2000 roentgens of X-ray

Test No.	No. of Eggs and Female Anaphes Used				Age of Eggs	No. and % of Eggs with BP[a]				No Adults Emerge				Ratio of BP/♀ Used	
	Control		Test			Control		Test		Control		Test		Control	Test
	Eggs	♀	Eggs	♀		No.	%	No.	%	♀	♂	♀	♂		
1	109	12	37	5	3	33	30	17	46	32	4	23	4	3.4	2.75
2	110	12	30	5	2	54	49	26	87	97	18	61	14	5.2	4.50

[a]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

appear to be somewhat better for Anaphes development than are the eggs from the X-rayed adults, although the difference between the two groups is small and possibly not significant.

There are some undesirable aspects to this technique for obtaining sterile eggs. The sexing of the beetles, although not difficult, is time consuming. There is always the possibility that a female is not virgin, especially if the beetles are not sexed immediately after being taken from diapause storage.

Egg Treatment

To obtain "sterile" CLB eggs by radiation treatment, it would probably be more convenient to treat the eggs directly after they have been obtained from untreated parents than to treat the parents. Egg treatment would eliminate time-consuming sexing.

According to Brennan (1967) exposure levels of 1000 rads of Beta radiation and above completely eliminate hatching of CLB larvae. Consequently, 250 eggs that were a maximum of 1-day old were treated with 1000 roentgens X-ray and another 250 were treated with 2000 roentgens of X-ray.

The eggs, 250 per plastic petri dish and stored at 40° F for 9 days before treatment, were treated with a Maximar 250-III machine at 310 roentgens per minute for 6.45 minutes while those treated with 1000 roentgens were treated for half that time. The eggs were stored at 40° F for 3 days

after treatment before being parasitized. The 250 control eggs were not subjected to X-ray. In this test, 13 female French Anaphes were placed on each group of 200 eggs (200 at 2000 r, 200 at 1000 r, and 200 without X-ray). One hundred eggs (50 from the two different treatments) were allowed to develop without parasites being present to see if any beetle larvae would develop and/or hatch. Table 28 gives the results of this experiment and Table 29 lists the results from the X-ray-treated eggs that had no parasites placed on them.

Table 28. The number of cereal leaf beetle larvae to hatch, number of parasitized eggs to develop black pupae, and the number of adult parasites to emerge from cereal leaf beetle eggs treated with 1000 and 2000 roentgens X-ray and untreated eggs (200 eggs for each test group)

	Untreated	1000 r	2000 r
No. cereal leaf beetle larvae to hatch	51	60	38
No. cereal leaf beetle larvae to develop[a]	19	17	43
No. eggs with nothing developing	69	56	65
No. eggs parasitized	61	67	54
No. female Anaphes	62	75	68
No. male Anaphes	21	17	32

[a]The number of cereal leaf beetle eggs developing to larvae but not hatching.

Table 29. The number of cereal leaf beetle larvae to develop and/or hatch from 50 eggs treated with 1000 roentgens and 50 eggs treated with 2000 roentgens X-ray that were not subjected to parasitization

	1000 roentgens	2000 roentgens
No. of cereal leaf beetle larvae to hatch	34	26
No. of eggs with cereal leaf beetle larval development[a]	10	17
No. of eggs with no signs of larval development	6	7

[a]The number of cereal leaf beetle larvae to develop but not hatch.

Since the hatch of the CLB larvae was fairly great at the levels of 1000 and 2000 roentgens (contrary to Brennan's 1967 results), further tests were conducted using 3000, 4000, and 5000 roentgens.

A total of 750 CLB eggs (1-day old and stored for 4 days before treatment) were treated with X-ray (250 eggs were treated at each level). The 250 eggs in each treatment (50 eggs per 1/3 section of cut glass slide) were in the same petri dish with the lid taped shut and moist filter paper on the bottom of the dish. Using the Maximar 250-III machine, the treatment levels were as follows: 3000 r - 9 minutes, 54 seconds at 330 r/minute; 4000 r - 12 minutes and 7 seconds at 330 r/minute; and 5000 r - 15 minutes and 9 seconds at

330 r/minute. From each of the treatments 50 eggs were not subjected to parasitization and 150 untreated eggs a maximum of 2-days old were used as controls (100 with parasites and 50 without). Five female Anaphes were used per 50 eggs. Table 30 presents the results of the treated and untreated CLB eggs not subjected to parasitization and Table 31 presents the results of those eggs subjected to parasitization.

Table 30. The number of cereal leaf beetle larvae to develop and/or hatch from untreated cereal leaf beetle eggs and from eggs treated with 3000, 4000, and 5000 roentgens X-ray that were not subjected to parasitization

	Untreated (50 eggs)	Treated (50 eggs each)		
		3000 r	4000 r	5000 r
No. CLB[a] hatched	39	0	0	0[b]
No. CLB[a] to develop but not hatch	3	11	6	16

[a]CLB = cereal leaf beetle larvae.

[b]One cereal leaf beetle larva had partially emerged but never completely escaped from the egg.

Table 31. Parasitization success of Anaphes flavipes in cereal leaf beetle eggs treated with 3000, 4000, and 5000 roentgens X-ray

	Untreated		3000 r (200 Eggs)	4000 r (200 Eggs)	5000 r (200 Eggs)
	100 Eggs	2x[a]			
No. BP[b]	68	136	144	108	89
Percent parasitism	68	68	72	54	44.5
No. female Anaphes	108	216	235	188	132
No. male Anaphes	22	44	43	35	19
Total Anaphes	130	260	278	223	151
No. BP[b] not to emerge	2	4	11	4	19
No. CLB[c] hatch	8	16	0	0	0
No. CLB[c] to develop but not hatch	3	6	4	11	14

[a]These values are two times the values obtained for 100 untreated eggs so they can more easily be compared with the test groups.

[b]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c]CLB = cereal leaf beetle larvae.

Ten F_1 females from each of the test groups to emerge were put on 50 unparasitized CLB eggs that were a maximum of 2-days old and had been stored at 40° F for 5 days before use. The females were mated for 1/2 hour and fed and watered for 1 hour before being used. The test was conducted to see if development from X-ray treated eggs affected the parasites that emerged. The results are presented in Table 32.

Table 32. Parasitization success of F_1 *Anaphes flavipes* females from cereal leaf beetle eggs treated with 3000, 4000, and 5000 roentgens of X-ray on untreated cereal leaf beetle eggs

	3000 r	4000 r	5000 r
No. BP[a]	33	44	40
No. females	28	99	75
No. males	40	17	16

[a]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

Discussion

The X-ray tests of eggs indicate that 1000 or 2000 r is not great enough to effectively retard the development of CLB larvae as seen from the number of larvae that developed from both the treated eggs exposed to parasites and those that were not exposed to parasites. These results seem to be contrary to the findings of Brennen (1967). He found that at and above 1000 rads, CLB larval hatch was completely eliminated. The reason for this discrepancy is not known unless it is the result of experimental error or due to the difference in the type of radiation used (Brennen used Beta radiation). There is the possibility that the discrepancies are the result of improper dosimetry. With the equipment Brennen used, the dosage given would have been for about 1/50th of a second; this short period of time may be difficult to control with the result that dosages Brennen gave were actually greater than he stated. In addition, the

dosimeter used with the Maximar machine has not been calibrated for sometime, consequently the dosages used may not have been exactly those calculated.

Although slightly fewer larvae hatched at the 2000 r level than at the 1000 r level there is very little difference in terms of the total number of larvae to develop and/or emerge. At levels of 3000 r and above larval hatch is completely eliminated and larval development is very much reduced.

In addition, all levels of X-ray used on eggs do not prevent the development of Anaphes. The use of Chi Square shows no significant difference in terms of the black pupal development between controls, 1000 r, and 2000 r of X-ray. Furthermore, the use of a two-way analysis of variance indicates no difference between the 3000 r, 4000 r, and 5000 r levels of X-ray in terms of the development of black pupae. The raw data suggests no difference between the 3000 r level and untreated eggs, but there may be differences with the higher levels of X-ray, especially at 5000 r. It appears that as the level of radiation used on the eggs increases from 3000 to 5000 r, there is a decrease in the number of eggs that develop black pupae.

Females emerging from eggs treated with 3000 r parasitized fewer untreated eggs and produced fewer offspring than those from 4000 r and 5000 r levels. Even so,

there probably is little difference in the effects of Anaphes development from eggs treated with 3000, 4000, and 5000 roentgens.

Placing more CLB larvae into the field population by the mass release of immature stages of Anaphes is of no real danger since any CLB larvae that are going to hatch, hatch and die in the lab before the releases occur. The danger is eliminated since it takes 4 to 7 days for CLB larvae to hatch, but 8 to 10 days for the development of the Anaphes black pupal stage.

To obtain sterile eggs for the development of Anaphes it is more economical to treat the eggs directly than to get them from unirradiated female CLBs mated with irradiated males. Although Anaphes develops from the eggs of irradiated parents, it is time-consuming to sex the parents before treatment and the females used must be virgin. It appears that in the direct application of X-ray to eggs, the 3000 r level of X-ray is best to obtain maximum parasite production and no CLB larval hatch.

FIELD RELEASES

The mass culturing of Anaphes has been conducted to provide sufficient numbers to attempt establishment in those areas where the CLB poses a threat to agriculture.

Techniques

There are essentially two means of field releasing Anaphes: either as adults or as black pupae. With one exception, all releases utilized the latter.

There are several reasons for the selection of the black pupal stage for field release. Black pupae, since they can be stored effectively for at least 2 weeks, need not be placed in the field at the time they reach the pupal stage; adults must be released almost immediately since they only live from 1 to 4 or 5 days in the lab. When adults are released almost invariably there are some that do not emerge the day of the release, but instead emerge a day or even 2 days later. By releasing the parasites in the black pupal stage, there is a much better chance that these stragglers will get into the field, especially when releases are made at other than daily intervals. When releasing adults mating can be ensured before the releases. However, mating appears to be no problem in the release of black pupae since field recoveries indicate that when the pupal stage is released,

mating does occur after emergence and one obtains a satisfactory sex ratio in the field (approximately 2.3 females to one male).

There have been two methods used in field releasing the black pupae: wooden stakes to which are taped the coverslips or glass slides with the parasitized eggs, and wooden stakes with milk cartons that shelter the parasitized eggs. The 1966 trials utilized the first method of release. The 1/3 sections of cut glass slides with the parasitized eggs were taped to one side of wooden stakes (23-3/4 x 1-1/2 inches) which in turn were placed in the ground at an angle of from 15° to 30° with the eggs on the lower side to protect them from the elements (see Figs. 12 and 16). From four to six sections of slides with 50 to 100 eggs each were placed on each stake (see Fig. 11). This method is unsuitable for release of large numbers of parasitized eggs since it is time consuming to tape each slide or cover slip to the stakes.

The second container was used in the spring and summer of 1967. The release containers, patterned after those used by the Niles lab, consisted of wooden stakes to which were attached two, 1 quart milk cartons held in place with tacks. A piece of plywood was placed inside each carton to form a shelf. Thus, each carton could hold six 50 x 12 mm petri dishes (three on the shelf and three on the floor of the carton). The stakes were placed in the ground

Figure 11 - Wooden stakes used for releasing Anaphes flavipes in the field. Note the sections of glass slides taped to the stakes. (Wheat field, Charles Bohn's farm, Galien, Mich., 1966.)

Figure 12 - Wooden stakes for the field release of Anaphes flavipes in position in Charles Bohn's wheat field (1966).

so the cartons were oriented parallel to the ground. The front of the cartons was kept closed (to keep rain out and the petri dishes in) with paper clips. The parasites escaped through a hole cut in the bottom of the front of the container (see Figs. 13 and 23).

The 1966 Release

The field releases were made in 1966 at four different sites in Michigan, one each in Hillsdale County and Kalamazoo County and two in Berrien County.

Hillsdale County

The release site in Hillsdale County (Section 31, T5S, R3W) was about 2 miles west of Jonesville on land worked by Walter Kochendorfer. The release, less than 100 feet from the nearest woodlot, was in an area of very low CLB numbers.

At this site, a pup-tent-shaped frame was placed in an oat field that was situated next to a wheat field. The frame, 4 feet long and 2 feet wide at the base and covered with window screen, had the legs and lower edges of the screen buried (Fig. 14). Cereal leaf beetles were swept up and confined in this cage (less than 100 beetles were swept up in several thousand sweeps from the field). The beetles were confined in the cage so oviposition would be concentrated. Three days later, 195 parasitized CLB eggs were placed in the cage which was then covered with fine-mesh cloth so the emerging parasites could not escape (Fig. 15).

Figure 13 - Milk carton release containers before being closed. In the second set of containers from the right in the front row, the wooden shelf in the top release container is visible. (Release site in Jackson County in 1967, looking south - oats.)

Figure 14 - The release cage used in Hillsdale County in 1966. Note the release stakes inside the cage and the woodlot in the background.

Figure 15 - The release cage in Hillsdale County in 1966 after fine white cloth was placed over the cage.

Ten days later, 183 parasitized eggs were released in the same field using the same techniques. Table 33 gives the dates and numbers of parasitized eggs released.

Table 33. The date, number of parasitized eggs, and location of Anaphes flavipes releases in 1966

Date	No. BP[a]					
			Berrien County			
			Bohn's		Tobler's	
	Jackson County	Kalamazoo County	Oat	Wheat	NE Corner	SE Corner
19 May	195	...	198	199	...	...
23 May	...	...	200	216	...	...
29 May	183	...	...	...	...[b]	...
4 June	...	...	...	...	250[b]	150[b] 80 BP
10 June	...	456	...	...	...	...
11 June	...	...	321	...	...	...

[a]BP = the number of parasitized cereal leaf beetle eggs released.

[b]These were adult Anaphes.

Kalamazoo County

The release site in Kalamazoo County was about 6 miles northeast of Vicksburg, on land owned by Earl Guyer (T3S, R10W, Sec. 22). The release was made in an area of low to medium CLB populations. It was made close to a dead tree near the center of an oat field about 1/4 mile from the nearest woodlot. On June 10, 456 parasitized eggs were released.

Berrien County

In Berrien County one of the release sites was about 2 miles SSE of Galien, 1/2 mile from the Indiana line, on land owned by Charles Bohn (T8S, R19W, Sec. 24). There were two different locations used here in the release of Anaphes. One location was in wheat just south of Olive Branch Road with a wooded area on the west side of the field (see Figs. 11 and 12). The other location was in oats about 1/4 mile south of the site just described (see Figs. 16 and 17). There was a woodlot about 25 yards away along the southern border of the field.

At the first site, 415 parasitized eggs were released in two releases 5 days apart. In the oats, 719 parasitized eggs were released at three separate times (Table 33).

Another site in Berrien County was also used. This site was about 2 miles SW of Galien, on land owned by Joe Tobler (T8S, R19W, Sec. 17). The releases were made at the NE and SE corners of an oat field near a woodlot. Emerged adults were released here, and females were observed ovipositing in CLB eggs shortly after release. The CLB population was high in this field and without our knowledge Joe Tobler sprayed with malathion 4 days after the parasite releases were made, leaving an unsprayed area about 50 feet in diameter around the release points.

Figure 16 - Release site in oats at Bohn's in 1966 (looking east). Note the stakes and the brush along the edge of the field.

Figure 17 - Charles Bohn's oat field in 1966 showing the woods along the south edge of the field (looking west). The circle marks the location of the release site.

1966 and 1967 Recovery Attempts

Recovery was not attempted in Jackson County in 1966 or 1967 because of the very low CLB population and because of the very small number of Anaphes released.

In Kalamazoo County on 1 July 1966 a single parasitized egg was recovered which contained three Anaphes. Very few eggs were present at this time. In 1967, one set of samples was taken from here. Twenty-five, 1 yard samples were taken 19 June from wheat at random within a radius of 25 feet from the 1966 release point. The 25 square yards of wheat plants yielded four eggs, three of which had already hatched when the samples were taken. The other egg had a CLB larva hatch in the lab.

In Berrien County in 1966, 4 days after the last release was made in Bohn's wheat field, 18 eggs were collected within 2 yards of the release point. Six eggs were parasitized, from which 13 female and three male parasites emerged beginning 3 June. In addition, 20 days after the last release, eggs in the same area were checked and six were taken to the lab because they appeared to be parasitized. The next day parasites began emerging with a total of six female and four male Anaphes emerging. No additional attempts at recovery at either site in Bohn's fields were made so as to leave the maximum number of parasites in the field for overwintering.

In 1967, wheat was growing where oats had been the previous year on Charles Bohn's farm. To assure the presence of beetles and eggs throughout the season at the 1966 release site, a strip of oats about 15 feet wide was planted running the length of the field on the east side.

Samples were taken at this site on 28 May, 15 June, 27 June, and 7 July, 1967. The samples consisted of 25, one yard strips of grain cut at random from the oat strip. The samples were placed in plastic bags after which the open ends were stapled closed. The samples were then placed in storage at 40° F until they were checked for eggs. Table 34 lists the results of this sampling.

Table 34. 1967 sampling results from Bohn's wheat field for overwintered *Anaphes flavipes*

Sample Date	Total No. of Eggs	No. of Eggs to Have Emergence Before Sample Checked	No. CLB[a] to Emerge After Sample Checked	No. of Eggs with No Emergence	No. of *Anaphes* to Emerge
28 May	277	139	91	47	0
15 Jun	46	6	8	32	0
27 Jun	217	125	40	52	0
7 Jul	53	47	4	2	0

[a]CLB = cereal leaf beetle larvae.

From the samples taken 27 June, 1967 one egg was found to have had three parasites emerge. These parasites were not Anaphes and were later identified as male Trichogramma minutum Riley, by Dr. B. D. Burks of the U.S.N.M. (The Niles laboratory has also recovered Trichogramma from Berrien County, but they have been identified as T. evanescens.)

Because of the malathion treatment no recovery attempts were made in Tobler's field in 1966 or 1967.

Discussion

The field releases made in 1966 indicated that Anaphes will parasitize CLB eggs when they are released in the field. Also, it is possible that more than one generation was obtained from the releases made in Charles Bohn's wheat in 1966. If the Anaphes that emerged from eggs collected 20 days after the last release in Bohn's wheat were not F_2, they were F_1 and would have parasitized CLB eggs to start the F_2 generation.

Although the sampling of 1967 indicated that no Anaphes overwintered, the results of the sampling cannot be considered conclusive evidence that Anaphes did not overwinter. It is entirely possible that the sampling missed adults or progeny of overwintering adults. They could have been missed either because of the sampling method that was used, or because only a small number of parasites survived or a combination of these factors.

The sampling did indicate that there may be a native parasite that will parasitize the CLB eggs. Trichogramma minutum and/or evanescens are known as egg parasites of a variety of insects. It is possible that this parasite may find the CLB eggs so satisfactory for its development that it will build its population to the point where it may be of benefit in the control of the cereal leaf beetle. Even if this does not occur, it does indicate that there are native insects that may eventually find the CLB a suitable host.

1967 Field Releases

Field release of Anaphes were made at three sites in Michigan in 1967. The first site was in Watertown Township (T5N, R3W, Sec. 4) of Clinton County (Fig. 18). The second site, in Jackson County, was on the farm of Harold Benn Jr. located northwest of Jackson in Sandstone Township (T2S, R2W, Sec. 16). (See Fig. 19.) The third release site was in Ross Township (T1S, R9W, Sec. 4 and 9) of Kalamazoo County at the Kellogg Gull Lake Farms of Michigan State University (Fig. 20).

During the 1967 release period approximately 92,690 parasitized CLB eggs were released. Clinton County received 31,070 parasitized eggs, Jackson County 31,320 parasitized eggs, and 30,300 were released at Gull Lake (Table 35). The releases utilized milk cartons attached to wooden stakes for the field placement of parasitized CLB eggs (see Figs. 13 and 23).

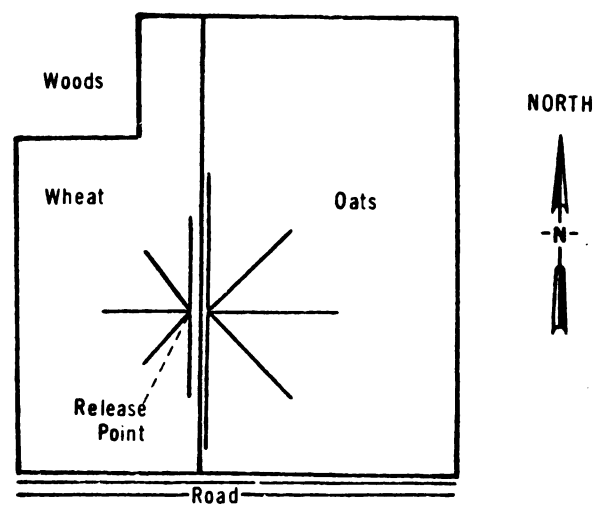


Figure 18 - Clinton County release site (1 mm = 15 feet).

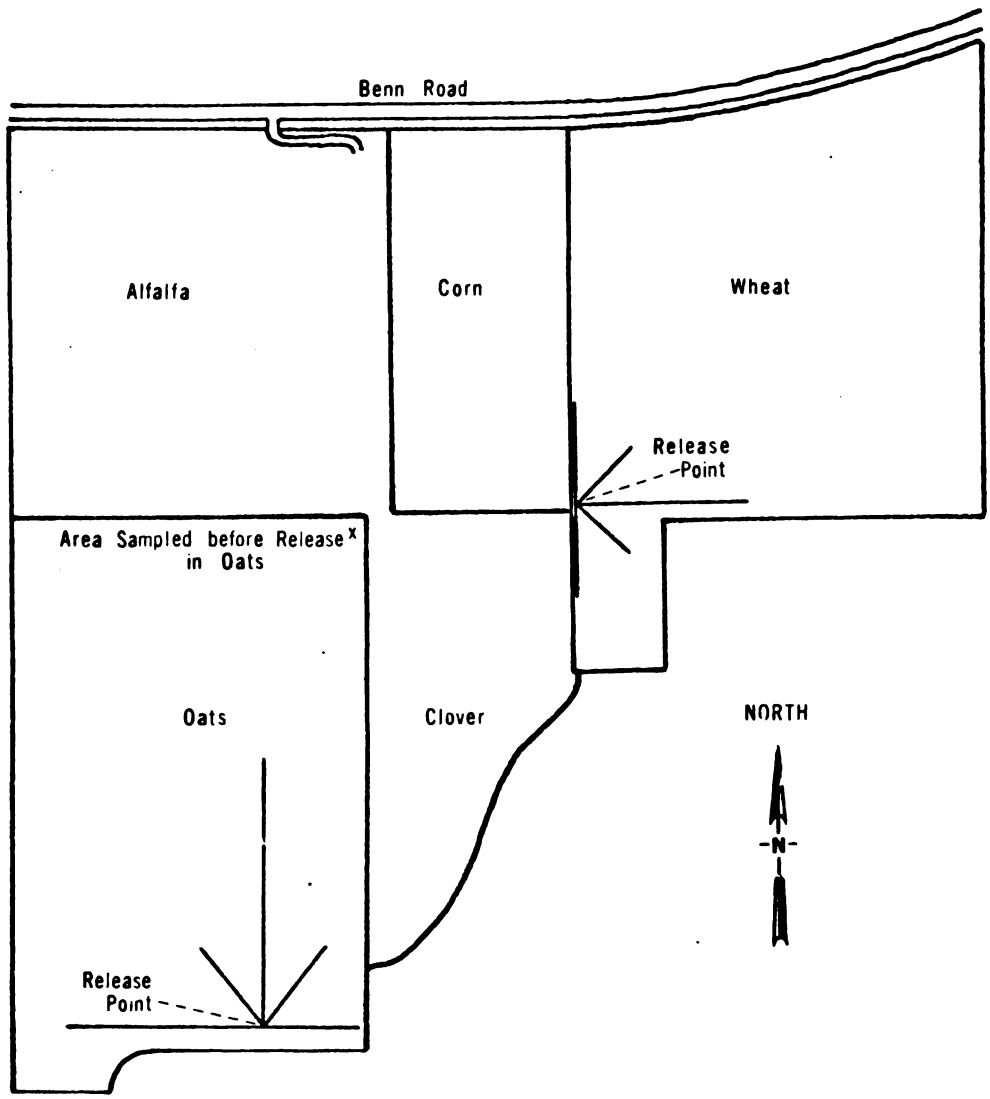


Figure 19 - Jackson County release site (1 mm = 15 feet).

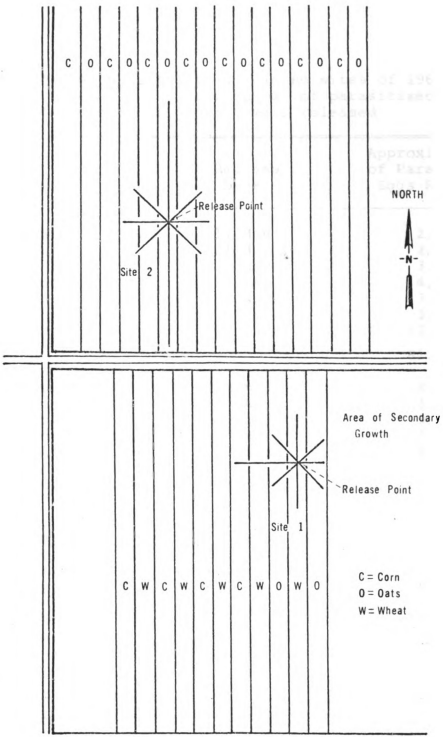


Figure 20 - Release sites at Gull Lake (1 mm = 15 feet).

Table 35. The *Anaphes flavipes* release sites of 1967, dates of release, and the number of parasitized cereal leaf beetle eggs that were released

Location	Release Date	Approximate No. of Parasitized Eggs Released
Clinton County	17 May	2,570
Clinton County	21 May	2,570
Clinton County	25 May	3,240
Clinton County	30 May	4,000
Clinton County	1 June	3,340
Clinton County	10 June	3,290
Clinton County	13 June	12,060
Total		31,070
Jackson County, Wheat	19 May	2,570
Jackson County, Wheat	23 May	2,510
Jackson County, Wheat	26 May	3,190
Jackson County, Wheat	29 May	3,960
Jackson County, Wheat	2 June	3,340
Jackson County, Oats	9 June	3,360
Jackson County, Oats	12 June	12,390
Total		31,320
Gull Lake, Site 1	19 May	2,570
Gull Lake, Site 1	23 May	3,290
Gull Lake, Site 1	26 May	3,190
Gull Lake, Site 1	29 May	4,000
Gull Lake, Site 1	4 June	3,440
Gull Lake, Site 1	9 June	3,290
Gull Lake, Site 2	16 June	10,520
Total		30,300
Grand Total		92,690

Samples were taken around the release points to determine the distance and directional dispersal of the parasites after release, and to obtain an indication of the egg density and parasitization rate.

The sampling pattern used was similar at all the sites. It basically consisted of four lines intersecting at the release point. Lines radiated to the north-south, east-west, northeast-southwest, and northwest-southeast. Along the north-south and east-west lines samples were taken 10, 40, 80, 120, and 180 feet north, south, east, and west of the release point; along the other lines samples were taken 5, 20, 60, 100, and 150 feet from the release point. Any samples taken at distances greater than the above were taken at 50 foot intervals.

Each sample taken during the survey period was approximately 1 square yard in size. After cutting, each sample was placed in a polyethylene bag, labelled, and stapled shut. This sometimes left the tops of tall plants exposed, but eggs are rarely found near the top or on the heads. Table 36 lists the range in total numbers of eggs taken per site and the average number of eggs per sample.

After cutting, the samples were taken to the lab where they were stored at 40° F until examined. The samples were checked for eggs and if any were found, they were removed and placed in petri dishes with moist filter paper, one petri dish for each sample. The eggs were first checked

Table 36. The number of cereal leaf beetle eggs taken at each Anaphes flavipes release site on the dates that samples were taken

Site	Date (1967)	Total Eggs Collected	Good Eggs[a]	Range in No. of Eggs/Sample		Average No. of Eggs/Sample	
				Total Eggs	Good Eggs[a]	Total Eggs	Good Eggs[a]
Clinton County, Wheat	1 June	21	20	0-5	0-5	.8	.8
Clinton County, Wheat	16 June	18	3	0-3	0-2	.7	.1
Clinton County, Oats	9 July	16	11	0-3	0-3	.5	.3
Jackson County, Wheat	2 June	25	12	0-6	0-4	1.0	.4
Jackson County, Wheat	12 June	42	13	0-8	0-3	1.6	.5
Jackson County, Oats	18 June	226	96	0-36	0-10	7.5	3.2
Jackson County, Oats	24 June	547	194	7-42	0-18	17.6	6.3
Jackson County, Oats	3 July	360	52	0-44	0-6	10.3	1.5
Jackson County, Oats	12 July	107	3	0-16	0-1	7.1	.2
Gull Lake, Site 1	4 June	811	792	0-132	0-132	20.8	20.3
Gull Lake, Site 1	19 June	595	370	0-100	0-70	15.2	9.5
Gull Lake, Site 1	23 June	426	194	0-97	0-37	25.0	11.4
Gull Lake, Site 1	1 July	702	198	3-112	0-30	43.9	12.4
(Voluntary Oats)[b]	14 July	70	7	8-62	0-7	...	...
Gull Lake, Site 2	19 June	772	281	0-112	0-43	33.6	12.2
Gull Lake, Site 2	23 June	2045	391	5-183	1-41	75.7	14.5
Gull Lake, Site 2	1 July	1118	90	8-84	0-10	41.4	3.3
Gull Lake, Site 2	14 July	190	4	0-36	0-1	17.3	.4

[a]Good eggs - Those eggs from which nothing had emerged at the time the sample was collected.

[b]The samples taken in voluntary oats were not square yard samples, but rather an uncounted number of oat plants taken at random.

for those that had had emergence before the samples were collected, and those found were counted and discarded. The remaining eggs (= "good eggs" in Table 36) were observed in the lab for the development of Anaphes. As the parasites developed, the number of parasitized eggs was counted, and after emergence the adults were sexed and counted. Samples were checked daily for emerged parasites, and every other day they were more closely checked for emerged CLB larvae, parasitized eggs, collapsed eggs or anything else that might be of interest. Table 37 lists the date, location, number of samples taken, and the number of samples with parasitized eggs.

Clinton County

In Clinton County, all releases were made in a wheat field with a small woodlot at the north end. There was an oat field adjacent to the east side of the wheat field but it was planted too late for any of the releases to be made there. Seven separate releases were made in Clinton County. Table 35 lists the releases and Figure 21 shows the sampling pattern used. This site was chosen primarily because of the low CLB population present (0-5 eggs/sq. yd.).

Parasitized eggs were recovered at a maximum distance of 40 feet north, south, and west of the release point. When the samples for 16 June were checked there was one egg in a sample taken 60 feet northwest of the release point that appeared to have had parasites emerge before the sample was

Table 37. Date, location, number of samples taken, and the number of samples containing *Anaphes flavipes* for the 1967 release sites

Release Site	Date Sampled	No. Samples	No. Samples with Parasites
Clinton County, Wheat	1 June	25	4
Clinton County, Wheat	16 June	25	0
Clinton County, Oats	9 July	35	0
Jackson County, Wheat	2 June	27	0
Jackson County, Wheat	12 June	27	1
Jackson County, Oats	18 June	31	6
Jackson County, Oats	24 June	31	17
Jackson County, Oats	3 July	37	14
Jackson County, Oats	12 July	15	2
Kalamazoo County:			
Gull Lake, Site 1, Wheat & Oats	4 June	39	5
Gull Lake, Site 1, Wheat & Oats	19 June	39	7
Gull Lake, Site 1, Oats	23 June	17	9
Gull Lake, Site 1, Oats	1 July	16	13
Gull Lake, Site 1, Voluntary oats in corn	14 July	2	1
Kalamazoo County:			
Gull Lake, Site 2, Oats	19 June	23	8
Gull Lake, Site 2, Oats	23 June	27	11
Gull Lake, Site 2, Oats	1 July	27	7
Gull Lake, Site 2, Oats	14 July	11	1

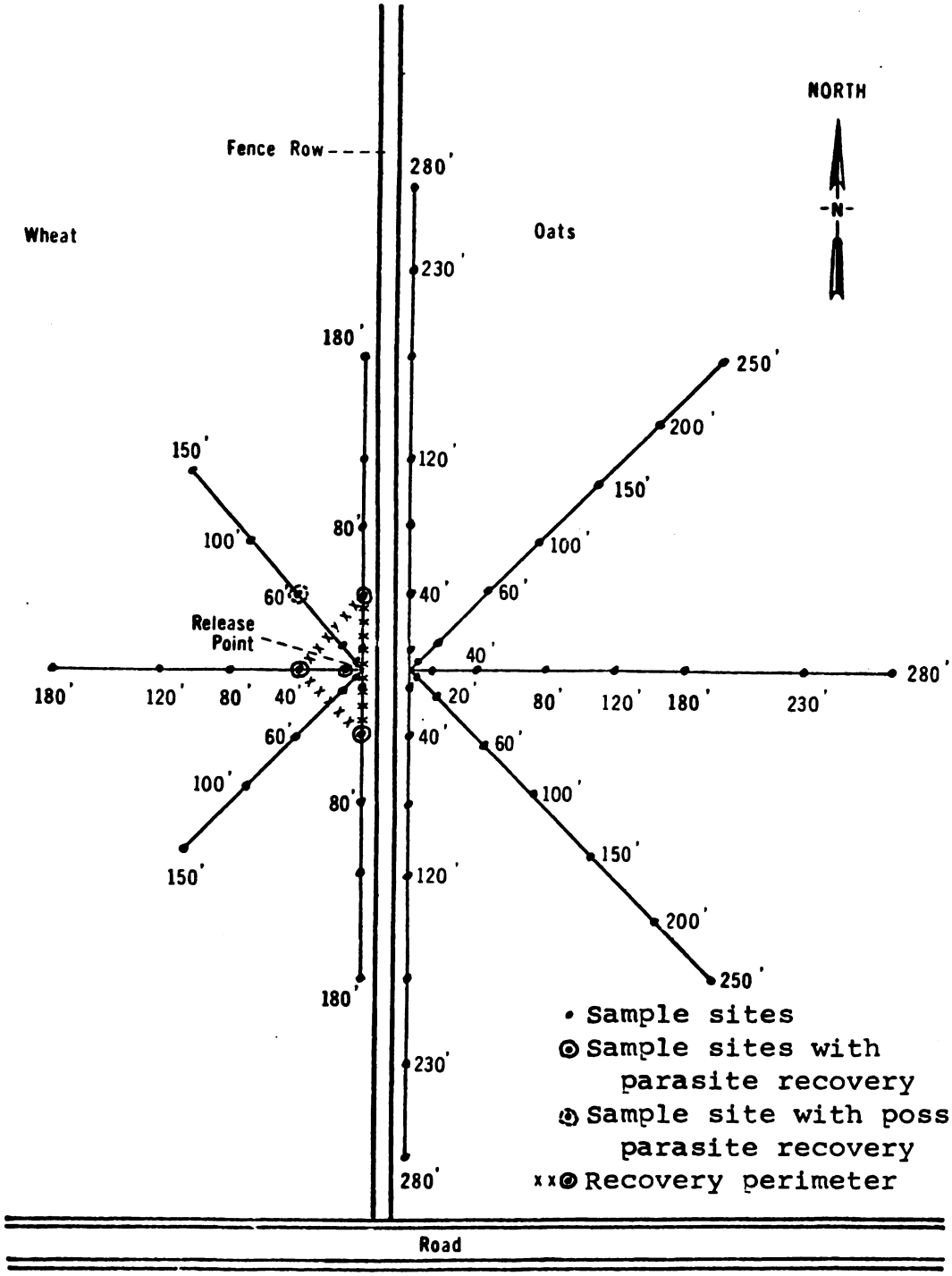


Figure 21 - Sample points and recovery perimeter of Anaphes for 1 June 1967 in Clinton County (1 cm = 40 feet).

checked. If this is correct then the maximum dispersal distance as shown by the samples was 60 feet. The circled sample locations in Figure 21 are those locations where parasites were recovered.

In addition, one set of 35 samples was taken from the oats on 9 July to see if Anaphes had moved into them. None of the 16 eggs collected showed signs of Anaphes.

The parasites that did emerge from the samples were first or second generation since the maximum development time from the original release to the time the adults emerged in the lab was about 20 days. In the lab, it might be possible to get two generations in this time period (this would require an average daily temperature of approximately 80^{o} F). It is difficult to determine whether these offspring are F_1 or F_2 due to the multiple releases made in this field.

Jackson County

The site at Jackson is actually two sites: a wheat field and an oat field. The first five releases were made in the wheat; once the oats were up and the beetles had moved into it the last two releases were made there. There is a woodlot about 290 feet south of the release point in the wheat. The west edge of the wheat field was bordered by clover and corn. On the west side of the clover was the oat field. The two release points were about 1225 feet apart. A list of the releases is found in Table 35.

The only sample from wheat in Jackson County to have parasites develop was taken 20 feet to the northeast of the release point. CLB oviposition was low in the wheat; consequently the number of eggs per sample ranged from 0 to 6 for 2 June and 0 to 8 for 12 June, 1967. In Figure 22 the circled sample site is where the parasitized egg was found.

Before any releases were made in the oat field ten, 1-yard strips of oats were taken from the northeast corner of the oat field opposite the release point in the wheat (Fig. 19). No Anaphes developed in any of the 64 eggs recovered, indicating that Anaphes may not have crossed from the wheat to the oats.

The point of release in oats was 180 feet from the east side of the field and 40 feet from the south edge. There is a woods and stream located south of the point of release (Figs. 13 and 19).

The maximum dispersal shown by the samples from the oat field of Jackson County is 430 feet directly north of the release point. In addition, a sample taken 280 feet to the west of the release point also had parasitized eggs. Figure 24 shows the sampling pattern used and the distribution of parasitized eggs. The circled sample sites in Figure 24 are those that contained parasitized eggs.

Little can be said about the dispersal of Anaphes in wheat on Benn's farm since only one sample contained parasitized eggs, but for oats several observations can be made.

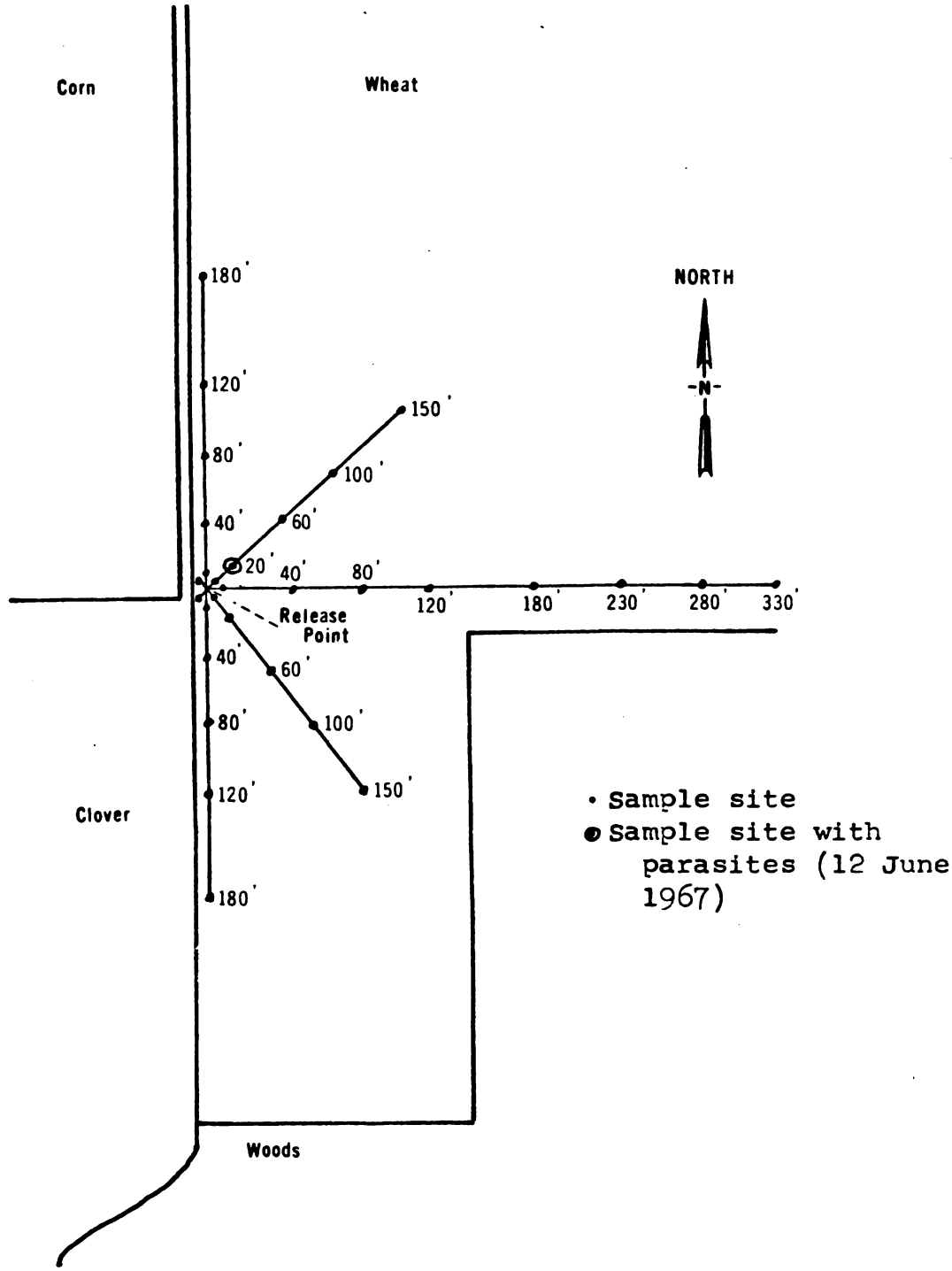


Figure 22 - Sample points in wheat of Jackson County (1 cm = 40 feet).

Figure 23 - The Jackson release site in oats, looking northeast. Note the release containers and the wooden stakes. The stakes mark the location of the sampling sites.

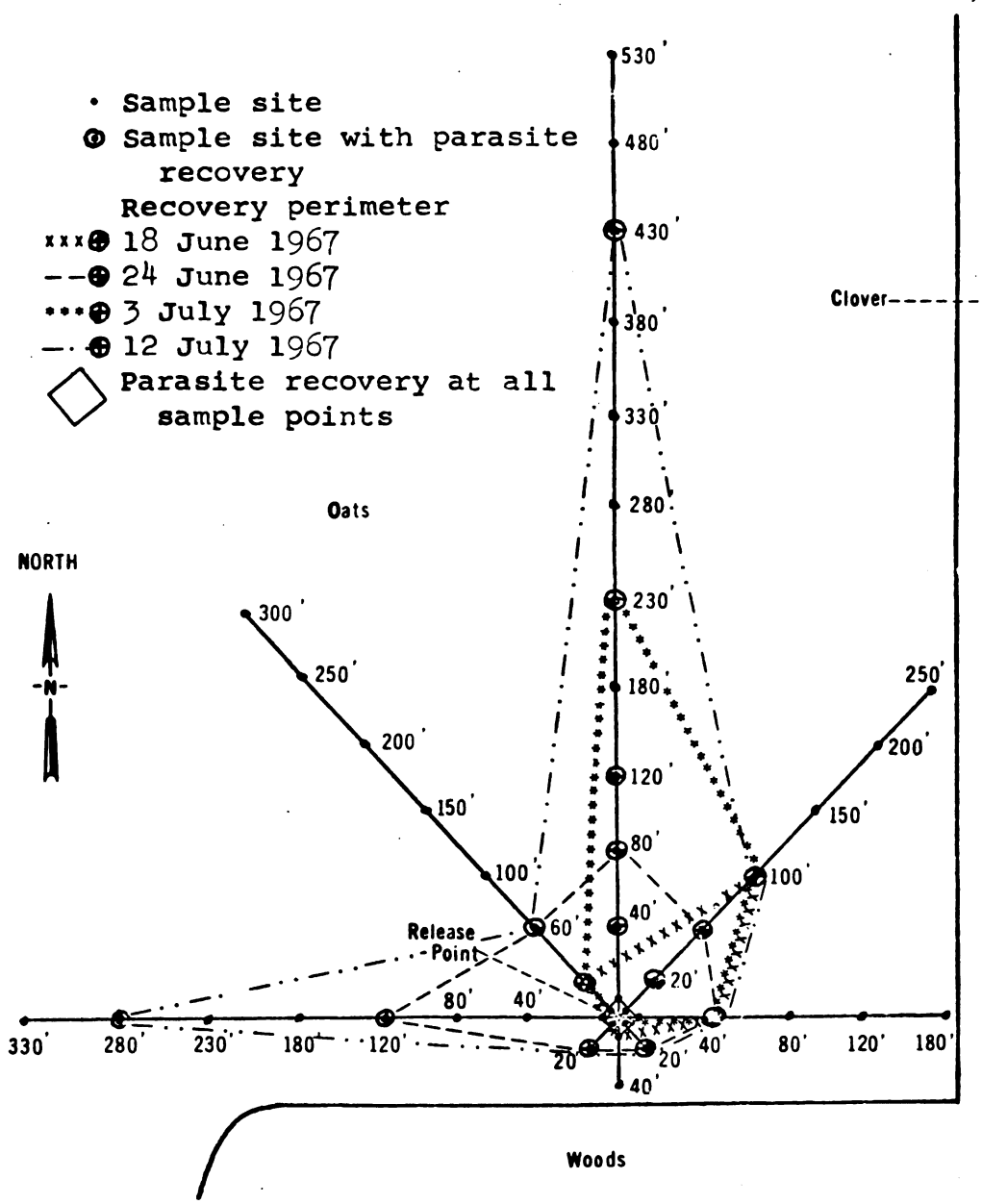


Figure 24 - Sample points and recovery perimeter of Anaphes in oats for Jackson County (1 cm = 40 feet).

Anaphes probably reached the woods to the south of the release site; if so, it should find an overwintering site if it overwinters as an adult.

The first samples taken from the oats in Jackson on 18 June indicated a general movement to the northeast but when sampled on 24 June, the dispersal indicated movement to the north, northwest, and west. On 3 July, the sampling indicated that northern dispersal had become predominant with no greater movement in the other directions than had occurred on 18 June. The samples of 12 July indicated that the parasite movement to the north was maintained and that movement to the west had again occurred. Since the samples of 12 July were taken at 50 foot intervals at greater distances than the previous samples, no samples were taken to the east and only one was taken to the northeast. Samples could not be taken any farther in these directions since the sampling was already near if not at the edge of the field. Consequently, nothing can be said for dispersal to the east or northeast for 12 July. For the distribution pattern of 12 July in Figure 24, the most distant sample sites that previously had Anaphes were used for the pattern to the east, northeast, northwest, and south.

Although samples were taken as far as 250 feet to the northeast and 300 feet to the northwest, the maximum distance that Anaphes was found to the northeast was 100 feet and only 60 feet to the northwest. If dispersal into the

woods to the south, southeast, or southwest occurred it was not detectable since the release was made only 40 feet from the south edge of the field.

In the releases made in the oats, it is difficult to separate the generations present in the field for several reasons: (1) the two separate releases were made 3 days apart, (2) parasite emergence usually ranges over several days so that all the parasites released probably did not emerge the same day (this also holds true for the succeeding generations), and (3) females parasitize eggs over a period of several days. With the above factors in mind, an attempt has been made to determine the number of generations recovered in the field.

Parasites emerging from the eggs recovered 18 June (Table 37) were F_1 since no more than 8 days had elapsed between recovery and the first release (parasites require 11 to 13 days to develop in the lab between 65° and 75° F). The adult parasites emerged in the lab 2 to 6 days after the samples were taken which means they were the equivalent of 9 to 5-day-old lab-reared parasites when collected in the field. Using the ratio,

$$\frac{9 \text{ days}}{8 \text{ days}} = \frac{11 \text{ days}}{X}, \quad X = 9.8 \text{ days}$$

where 9 days = lab equivalent of the parasitized eggs when recovered from the field.

8 days = length of time the eggs were actually in the field, assuming the eggs were parasitized the day after the first release (10 June).

11 days = the normal development time for parasites in the lab at approximately 75° F.

X = the estimated number of days for Anaphes to emerge in the field.

F_1 development in the field for the first release required about 10 days. Thus the F_1 should have begun emergence in the field about 20 June.

The F_1 development is somewhat shorter than lab development at 75° F but the temperature in the field (Table 38--it is assumed the temperature for Jackson is similar to that of the Benn farm which is 6 miles from Jackson), averaged 75° from 10 to 20 June and for the first 7 days the average was 79° F. The temperature in the field probably explains the rapid development.

The parasites that emerged from eggs recovered 24 June required from 1 to 7 days to emerge in the laboratory. Since it was calculated that the F_1 of the first release emerged about 20 June, those eggs that required 7 days to develop were the offspring of the F_1 that emerged 20 June. From the ratio

$$\frac{4 \text{ days}}{4 \text{ days}} = \frac{11 \text{ days}}{X}, \quad X = 11 \text{ days}$$

where 4 days = both the lab equivalent age of the parasitized eggs when recovered from the field and the total amount of time spent in the field (20 June to 24 June).

11 days = the normal development time for parasites in the lab at approximately 75° F.

X = the estimated number of days for Anaphes to emerge in the field.

one finds that the F_2 would have emerged 11 days after 20 June or about 1 July.

Table 38. The minimum, maximum, and average daily temperatures for the city of Jackson, Michigan (9 June to 17 July, 1967)[a]

Date	Temperature			Date	Temperature		
	Min.	Max.	Aver.		Min.	Max.	Aver.
June 9	69	86	78	June 27	55	81	68
June 10	66	85	76	June 28	62	69	66
June 11	69	82	76	June 29	61	75	68
June 12	68	89	79	June 30	60	88	74
June 13	66	89	78	July 1	70	89	80
June 14	67	90	79	July 2	56	77	67
June 15	72	92	82	July 3	54	71	63
June 16	70	92	81	July 4	53	67	60
June 17	62	75	69	July 5	52	70	61
June 18	58	76	67	July 6	50	79	65
June 19	50	76	63	July 7	57	82	70
June 20	58	83	71	July 8	63	86	75
June 21	55	73	64	July 9	72	87	80
June 22	60	76	68	July 10	72	80	76
June 23	58	80	69	July 11	66	88	77
June 24	60	86	73	July 12	60	84	72
June 25	54	68	61	July 13	49	73	61
June 26	52	76	64	July 14	49	65	57

[a]Data from Preliminary Climatological Data for Jackson, Michigan from the U.S. Weather Bureau.

The samples of 3 July required 1 to 7 days to emerge in the lab, thus in the field the parasites when recovered were the lab equivalent of 10 and 4 day old parasites. The eggs that were the equivalent of 10 day old lab-reared eggs were the F_2 that were to emerge 1 July. Using the ratio,

$$\frac{10 \text{ days}}{13 \text{ days}} = \frac{11 \text{ days}}{X}, \quad X = 14.3 \text{ days}$$

one finds that the F_2 required about 14 days to develop and not 11 days, so that they emerged about 4 July.

The F_1 of the 12 June release emerged about 25 June. This date is obtained from the ratio:

$$\frac{10 \text{ days}}{11 \text{ days}} = \frac{11 \text{ days}}{X}, \quad X = 12.1 \text{ days}$$

Since the temperatures (see Table 38) present in the field during the development of the F_2 for both the first and second releases are similar it is assumed F_2 development of the second release also required about 14 days, so they would have emerged about 9 July 1967.

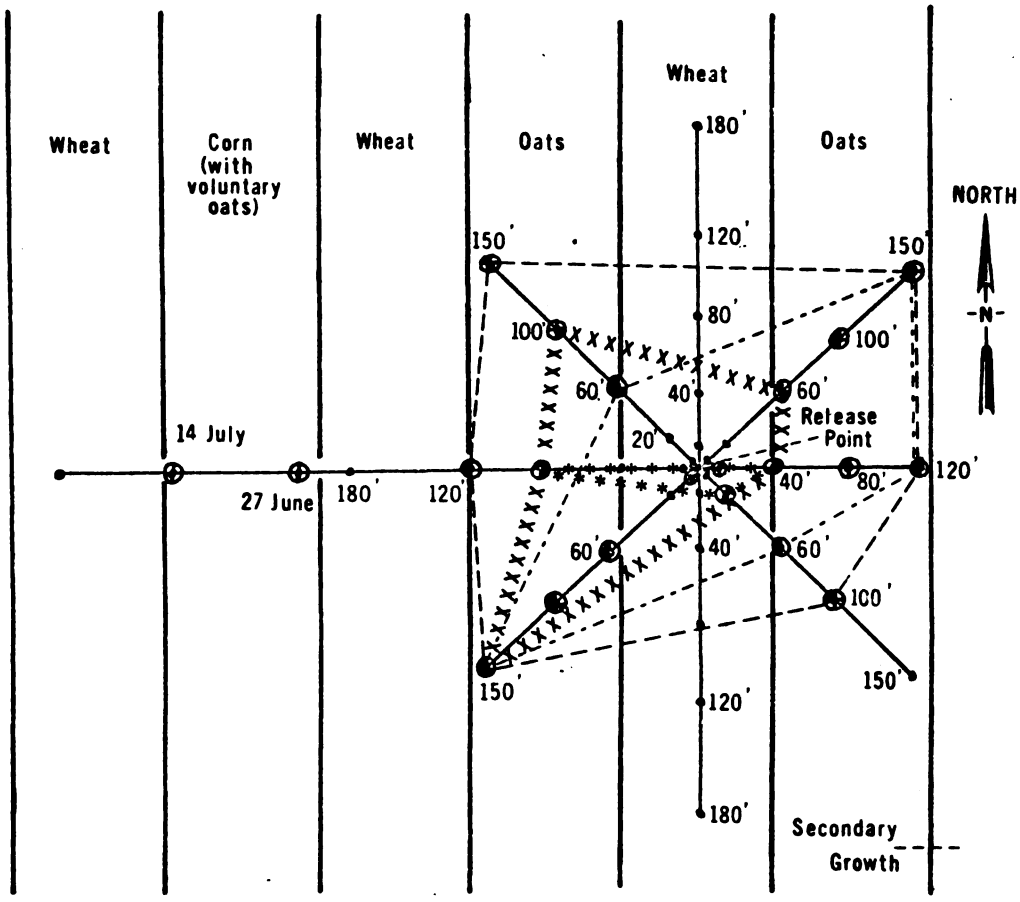
In addition, the samples of 12 July indicate the possibility of a third generation. The parasitized eggs required 3 days to emerge in the laboratory; hence, if parasitized by the F_2 that emerged 4 July, these would be F_3.

Gull Lake (Kalamazoo County)

Site 1.--Site 1 was bordered on the east side by an area of secondary plant growth (Figs. 20 and 25). Next to this growth was a strip of oats about 80 feet wide, bordered on the west by a strip of wheat 80 feet wide. The release point in the center of the wheat strip was approximately 350 feet south of the road (B Avenue) which forms the north boundary of the field. On the west side of the wheat strip was a strip of oats after which came alternating 80 foot strips of wheat and corn. Six releases were made at this site. Table 35 presents a list of the releases.

In the first two sets of samples taken from site 1 both the wheat and oats were sampled. After 19 June, only the oat strips were sampled because of the lack of eggs due to maturity of the wheat and movement of beetles from wheat to oats. In the samples of 4 June there was less than one egg per square yard of wheat and for 19 June there were only 13 eggs in 22, one square yard samples. Figure 25 shows the sampling pattern and dispersal of Anaphes at this site.

At site 1 parasitized eggs were found in samples taken 150 feet northwest, northeast, and southwest from the release point. In addition, samples taken from voluntary oats in the corn strip on a line west of the release point showed that the parasites had crossed into the corn and were on both the east and west edge of the corn strip. This means that the parasites crossed an 80 foot strip of oats,



• Sample site
⊙ Sample site with parasite recovery
Recovery perimeter
**⊕ 4 June 1967
xx⊕ 19 June 1967
---⊕ 23 June 1967
– –⊕ 1 July 1967

Figure 25 - Sample points and recovery perimeter of Anaphes on the south side of B Avenue (Site 1) Gull Lake, Michigan (1 cm = 40 feet).

then crossed an 80 foot strip of wheat (only three unparasitized eggs were found in one square yard of this wheat on 4 June and two on 19 June), and also crossed 80 feet of corn (height of the corn was not recorded). The total distance covered was 275 feet. The circled sample sites in Figure 25 are those from which parasitized eggs were recovered. The lack of Anaphes recovery from wheat on 19 June was undoubtedly due to the low number of eggs present in the wheat. The sampling showed that Anaphes moved out into those areas where eggs were present (in the oats). This is strikingly demonstrated in Figure 25 by the total lack of recoveries in wheat on the N-S sampling axis.

Sampling was stopped at the end of the diagonals originally because the extent of Anaphes dispersal had not been anticipated at the time sampling began. In addition, since sampling in wheat was discontinued after the second set of samples was taken, the diagonals were not extended even though Anaphes was found at the end of the southwest diagonal (150 feet from the release point). Furthermore, there were no other oat strips west of the diagonals into which to extend the sampling. The recoveries made in the corn were not placed in the dispersal pattern since these were separate samples taken by themselves, with no other samples taken at Site 1 at that time.

The number of generations to develop at Site 1 cannot be calculated because the adults of the six releases

overlap one another and are spread over 22 days at 3 to 6 day intervals.

Site 2.--After the beetles left the wheat and moved into the oats, the last release was made on 16 June on the north side of B Avenue which separates the fields. This is called Site 2. Here there were only corn and oat strips about 80 feet wide. The release was made in the center of an oat strip about 500 feet north of B Avenue which serves as the south boundary of the strips. Figure 26 shows the sampling pattern used.

Two of the samples taken here on 19 June were in the oat strips on either side of the adjacent corn strips as were six of the samples taken on 23 June (three in each strip). The 11 samples taken 14 July were at greater distances north and south of the release point than were the earlier samples.

One sample taken 1 June from Site 2 showed that parasites had crossed the adjacent 80 feet of corn and moved into the next oat strip. This sample was taken 180 feet west of the release point. The distance included about 40 feet of the oat strip where the release was made, 80 feet of corn and 60 feet of the next oat strip. In addition, one sample taken on 14 July that was 430 feet south of the release point had parasites develop. With the exceptions of the recoveries of Anaphes 180 and 430 feet from the release point all recoveries were within 20 feet of the release

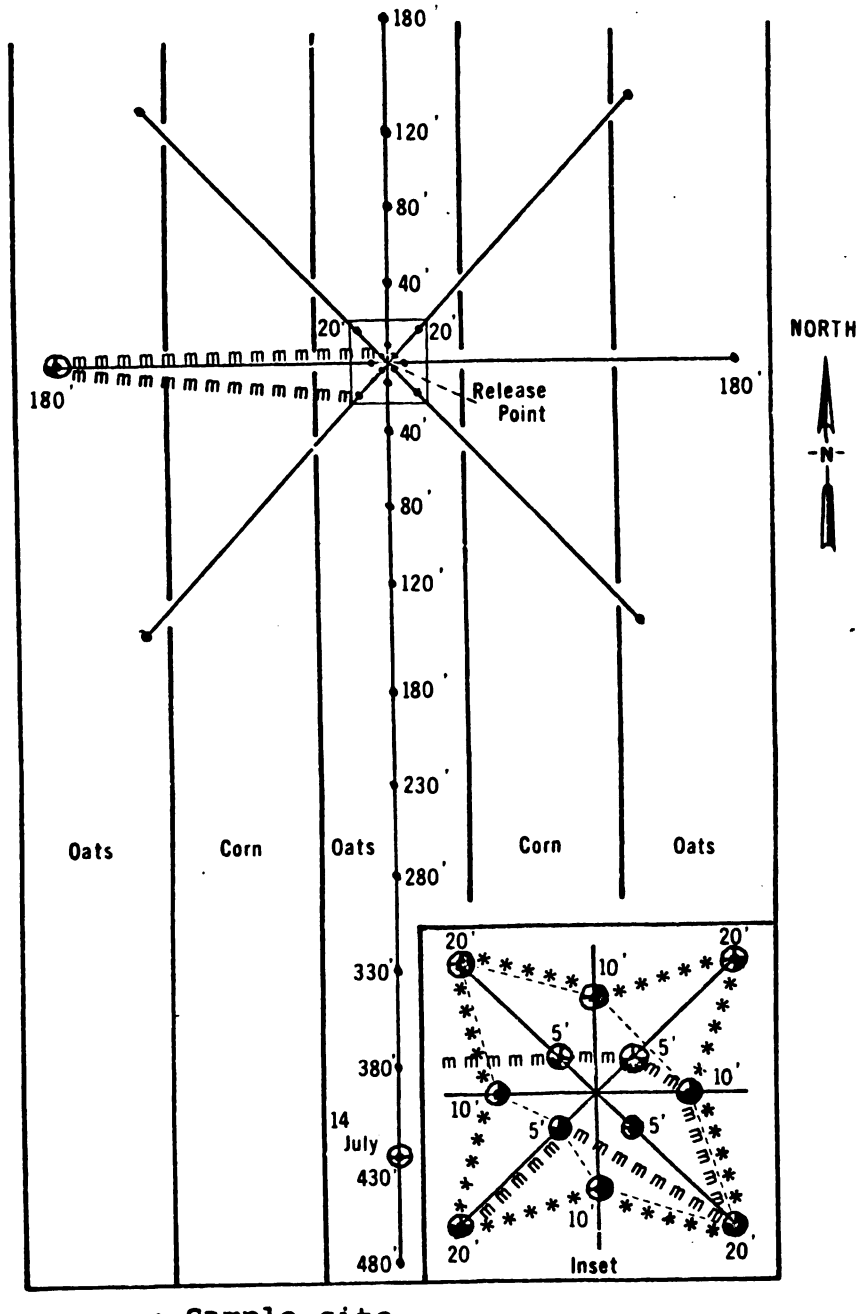


Figure 26 - Sample points and recovery perimeter of Anaphes on the north side of B Avenue (Site 2) Gull Lake, Michigan (1 cm = 40 feet).

point. The circled sample sites of Figure 26 are those from which parasites were recovered.

The parasites emerging from samples taken 19 and 23 June were F_1 since a maximum of 7 days had passed since the parent parasites were placed in the field as black pupae and the samples of 23 June were taken. Since one egg of the 1 July samples had Anaphes emerge the day they were first examined, it appears that development of the F_1 required about 14 days. Temperatures were similar from 16 June to 1 July and from 2 July to 14 July (Table 39), thus development times for the parasites recovered 14 July should be about 14 days, making the parasites recovered 14 July F_2.

Parasitization Rates

Table 40 lists the parasitization rates for the various release sites on the dates the samples were taken. Two different rates are given. In one, the parasitization rate is based on the total number of eggs found in the samples. The other rate is based on the number of good eggs which are defined as those that had not hatched or had anything emerge by the time the samples were collected.

When one looks at the parasitization of good eggs from oats at all the release sites, one finds there is a steady increase in the rate from the first set of samples to the last set. It is not at all obvious when one uses the total egg counts, but here one is unable to say definitely

Table 39. The minimum, maximum, and average daily temperatures for Gull Lake in Kalamazoo County, Michigan (16 May to 14 July, 1967)[a]

Date	Temperature			Date	Temperature		
	Min.	Max.	Aver.		Min.	Max.	Aver.
May 16	34	62	48	June 15	71	88	80
May 17	43	69	56	June 16	72	88	80
May 18	48	75	62	June 17	66	83	75
May 19	54	75	64	June 18	57	77	67
May 20	38	64	51	June 19	50	76	63
May 21	38	68	53	June 20	61	80	71
May 22	39	62	50	June 21	55	79	67
May 23	36	60	48	June 22	57	77	67
May 24	47	77	62	June 23	56	80	68
May 25	51	83	67	June 24	67	82	75
May 26	46	82	64	June 25	50	70	60
May 27	57	81	69	June 26	51	78	65
May 28	54	76	65	June 27	54	80	67
May 29	..[b]	..[b]	..[b]	June 28	59	79	69
May 30	41	70	56	June 29	60	75	68
May 31	44	68	56	June 30	60	83	72
June 1	45	78	62	July 1	65	85	75
June 2	46	80	63	July 2	61	84	73
June 3	51	86	69	July 3	64	75	70
June 4	51	82	66	July 4	48	70	59
June 5	58	85	72	July 5	46	68	57
June 6	63	79	71	July 6	50	78	64
June 7	64	75	70	July 7	54	79	67
June 8	65	82	74	July 8	64	82	73
June 9	67	82	74	July 9	67	82	75
June 10	65	83	74	July 10	69	80	70
June 11	66	81	74	July 11	65	85	75
June 12	66	88	77	July 12	62	82	72
June 13	66	87	77	July 13	57	77	67
June 14	65	86	76	July 14	51	67	59

[a] Record of Climatological Observations, W. K. Kellogg Biol. Station, Kalamazoo County, Michigan, Jan. to Dec., 1967, U.S. Weather Bureau.

[b] Temperatures not given.

Table 40. The number of female and male Anaphes flavipes to emerge from parasitized field collected eggs and the percent parasitization of the eggs from the samples taken in Clinton County, Jackson County, and at Gull Lake in 1967

Site	Date	Total No. Eggs	No. Good Eggs[a]	No. Eggs with BP[b]	Percent Parasitization: Total Eggs	Percent Parasitization: Good Eggs[a]	No. ♀	No. ♂	♀:♂ Ratio
Clinton County	6-1-67	21	20	5	23.8	25.0	7	2	3.5:1
Clinton County	6-16-67	18	3	0	0	0	0	0	.....
Clinton County	Total	39	23	5	13.0	21.7	7	2	3.5:1
Jackson County, Wheat	6-2-67	25	12	0	0	0	0	0	.....
Jackson County, Wheat	6-12-67	42	13	1	2.4	7.7	...[c]	...	.....
Jackson County, Wheat	Total	67	25	1	1.5	4.0	...	...	.....
Jackson County, Oats	6-18-67	226	96	11	4.9	11.4	8	3	2.7:1
Jackson County, Oats	6-24-67	547	194	56	10.3	28.9	107	44	2.4:1
Jackson County, Oats	7-3-67	360	52	30	8.3	57.7	51	19	2.7:1
Jackson County, Oats	7-12-67	107	3	2	1.9	66.7	2	1	2.0:1
Jackson County, Oats	Total	1240	345	99	8.0	28.7	168	67	2.5:1
Gull Lake, Site 1, Oats & Wheat	6-4-67	811	792	6	.007	.008	9	2	4.5:1
Gull Lake, Site 1, Oats & Wheat	6-19-67	595	370	76	11.1	20.5	118	48	2.5:1
Gull Lake, Site 1, Oats	6-23-67	426	194	57	13.4	29.4	62	28	2.2:1
Gull Lake, Site 1, Oats	7-1-67	702	198	140	19.9	77.8	261	91	2.9:1
Gull Lake, Site 1	Total	2534	1554	279	10.6	17.4	450	169	2.7:1
Gull Lake, Site 2, Oats	6-19-67	772	281	37	4.8	13.2	61	33	1.8:1
Gull Lake, Site 2, Oats	6-23-67	2045	391	69	3.4	17.7	81	61	1.3:1
Gull Lake, Site 2, Oats	7-1-67	1118	90	19	1.7	21.1	22	16	1.4:1
Gull Lake, Site 2, Oats	7-14-67	190	4	1	.005	25.0	1	0	1.0:1
Gull Lake, Site 2	Total	4125	766	126	3.5	16.4	165	110	1.5:1
Grand Totals		8005	2713	510	6.3	18.8	790	348	2.3:1
Clinton County, Oats[d]	7-9-67	16	11	0	0	0	0	0	.....

[a]Good eggs are those cereal leaf beetle eggs that had no emergence when the samples were first checked.

[b]BP = the number of parasitized cereal leaf beetle eggs that developed black pupae.

[c]No emergence occurred.

[d]The values for this location are not included in total values since there were no releases in this field.

how many eggs had previously had CLB larvae hatch and how many had parasites emerge.

In actuality, the real parasitization rate probably lies somewhere between the 6.3% "total egg rate" and the 18.8% "good egg rate." It is probably higher than the total egg rate since some of the spent eggs may have had Anaphes develop. If Anaphes developed from any of the spent eggs than the rate would be higher. Likewise, the true rate is probably lower than the good egg rate since it does not consider any of the eggs that had emergence before the samples were checked.

Summary

The sampling of 1967 showed that Anaphes can spread into adjacent strips of oats and wheat when the strips are as wide as 80 feet. It also showed that barriers of 80 foot wide corn strips can be crossed by Anaphes at least when voluntary oats are present in the corn. The sampling also showed that Anaphes can move at least 430 feet from the release point in one season. In addition, it was found that at least two and possibly three generations of Anaphes can be recovered from the field.

The 1967 release containers proved to be far superior to the stakes used in 1966 for releasing Anaphes as black pupae for several reasons: (1) they can be used more than once, (2) the placement of the parasitized eggs in the field is relatively easy and quick, (3) the containers protect the

eggs from the elements better than the stake method and (4) the carton's confined space probably allows more opportunity for mating by temporarily confining the emerging parasites together in closer quarters.

Black pupal release has several advantages over adult releases. The black pupae do not need to be placed in the field at the time of development as is true with adult releases, so when adverse weather conditions prevail on the release date, the pupae can be stored at 40° F until more favorable conditions occur. In addition, by releasing pupae, any stragglers that emerge a day or two later than the majority of the parasites will also get into the field; this is especially true if the releases are made at other than daily intervals.

SUMMARY AND DISCUSSION

In the mass culturing of Anaphes flavipes there are two basic techniques: removing CLB eggs from plants upon which they are laid or leaving the eggs on the plants. There is no difference between the two methods as evidenced by the number of parasitized eggs to develop per female used.

Although no difference occurred between the basic techniques of rearing Anaphes, at this time the disadvantages outweigh the advantages of the non-removal of CLB eggs from plants. The number of eggs within the plastic boxes (approximately 600 cubic inches in size) equals the number of eggs contained in three or four, 50 mm x 12 mm petri dishes used when eggs are removed from plants for parasitization. In other words, the containers require about 94 times as much space as do the petri dishes. In addition, the lack of an accurate method of estimating the number of eggs present in the plastic boxes and globes poses much of a problem to their use. Consequently, the use of petri dishes (removal of eggs from plants) is probably the better technique for mass rearing Anaphes even with the time consuming disadvantages of egg removal. When a technique for non-manual removal of eggs is developed the use of petri dishes will be far superior to the use of the larger mass culture containers.

With the use of petri dishes for rearing Anaphes an apparatus for collecting emerging adults has been developed. With the device about 93% of the emerging females are collected. Only 70% of the males to emerge are collected indicating that they may not be as attracted to light as are females. It is also possible that the males at emergence remain in the vicinity to mate with emerging females.

In the storage of unstung CLB eggs there appears to be a period of time after which they are no longer suitable for Anaphes development. At 40° F this appears to be approximately 6 to 8 weeks. It was originally believed that the presence of fungus on stored eggs was responsible, but tests with fungicide-treated eggs proved fungus did not cause the lack of egg suitability after 6 to 8 weeks of storage. With the use of gas atmospheres for CLB egg storage at 40° F the 6 to 8 week critical period is not increased, but neither is it decreased. At 14° F storage in gas atmospheres, development of either CLB larvae or Anaphes does not occur after 30 days of storage.

Although storage of unstung CLB eggs in various gas atmospheres has proved to be no better than storage in air at 40° F it does not mean that this technique will not prolong storage life of eggs. Since storage in gas at 40° F and storage in air at 40° F were similar perhaps further testing with other proportions and/or gases may prove successful. In the case of fruit and vegetable storage in

controlled atmospheres, conditions favorable for one fruit or vegetable may not be favoralbe for another or even for varieties of the same fruit or vegetable. Thus, this technique is potentially useful for long term storage of insects, and further tests should be conducted, not only with different gases and proportions, but also at temperatures other than 14° F and 40° F.

Parasitized eggs also are storable. Eggs in the black pupal stage can be stored for at least 7 weeks without eliminating viable sperm or mating of the resulting adults. However, after 2 to 4 weeks of storage, the resulting adults appear to be less active than those emerging from unstored eggs.

In parasitized eggs stored 2, 4, 6, and 8 days after parasite introduction, it was noted that as storage time increased, more and more parasitized eggs failed to have emergence. One of two reasons may explain the failure to emerge: (1) the parasites were dead or (2) they had gone into diapause. Since the parasitized eggs collapsed without anything emerging, it is not known whether the parasites would have emerged.

Anaphes can develop from CLB eggs laid by untreated female CLBs previously mated with males treated with 2000 roentgens X-ray. In addition, Anaphes develops from CLB eggs treated with as much as 5000 roentgens X-ray. Of treated eggs, those treated with 3000 roentgens appear best

for maximum parasite production and no CLB larval hatch. Furthermore, it is much easier working with eggs treated directly with X-ray than those eggs resulting from treated adults. With the direct treatment of eggs, the sexing of beetles is eliminated as well as the need for virgin females to be mated with the treated males (virgin females are needed to eliminate the possibility of previous matings with untreated males).

The 1966 field release of Anaphes indicated that it would develop in the field when released. Also, recoveries showed that when parasites are released as black pupae, mating does occur in the field.

Sampling in 1967 of 1966 release sites did not reveal the presence of overwintered Anaphes, but this cannot be construed to mean they did not overwinter. It is possible that too few survived the winter to be recovered in the sampling.

The 1967 release results indicated that Anaphes will disperse at least 430 feet from the release point in one season. Furthermore, the release showed that alternating 80 foot-wide rows of wheat and oats did not prevent the movement of Anaphes. It was also found that 80 foot-wide rows of corn did not act as a barrier to Anaphes, partly due to the presence of CLB eggs on voluntary oats in the corn.

Currently, there is in use at the Niles laboratory techniques that permit the production of sufficient parasites for field release in an attempt to establish Anaphes flavipes as a biological control agent of the cereal leaf beetle. It now remains to be seen whether the conditions present in those localities where Anaphes releases have been made are sufficient for its establishment. The location of its overwintering site(s) is the most important single piece of information needed to better estimate whether establishment of Anaphes will be possible (especially if an alternate host is necessary).

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