THESIS

THE EFFECTIVENESS OF TETRASTICHUS TRIOZAE BURKS (HYMENOPTERA: EULOPHIDAE) AS A BIOLOGICAL CONTROL AGENT OF PARATRIOZA COCKERELLI (SULC.) (HOMOPTERA: PSYLLIDAE) IN NORTH CENTRAL COLORADO

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY TIMM E. JOHNSON ENTITLED THE EFFECTIVENESS OF TETRASTICHUS TRIOZAE BURKS (HYMENOPTERA: EULOPHIDAE) AS A BIOLOGICAL CONTROL AGENT OF PARATRIOZA COCKERELLI (SULC.) (HOMOPTERA: PSYLLIDAE) IN NORTH CENTRAL COLORADO BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT

THE EFFECTIVENESS OF *TETRASTICHUS TRIOZAE* BURKS (HYMENOPTERA: EULOPHIDAE) AS A BIOLOGICAL CONTROL AGENT OF *PARATRIOZA COCKERELLI* (SULC.) (HOMOPTERA: PSYLLIDAE) IN NORTH CENTRAL COLORADO

This study was undertaken to determine some of the factors affecting the value of the parasite, *Tetrastichus triozae* Burks, as a control agent of the potato psyllid, *Paratrioza cockerelli* (Sulc.), in the potato growing areas of Weld and Morgan counties.

Field observations revealed that the parasite appeared after the spring psyllid infestation had declined and that the parasite population had declined by the time the fall psyllid infestation had built up again. Parasite pupal mortality ranged from 38-100% in the field populations. The time of appearance and high pupal mortality appeared to reduce the effectiveness of the parasite.

In the laboratory, the parasite and psyllid cycles were synchronized in relation to time of development. Because the parasite attacked only fourth and fifth instar psyllid nymphs, the parasite could not prevent psyllid yellows from killing the potato plants. This makes mass releases economically unfeasible in commercial fields. However, the parasite was efficient in the laboratory at controlling the psyllid populations indicating that the parasite might be of value as a biological control agent in the overwintering areas.

Parasite dispersal was very rapid when the distance between host plants was 5 feet or less; distances of 7 -30 feet greatly reduced dispersal. This may account for localization of the parasites. The cause of the high pupal mortality was not ascertained, but disease and predators may have been partially responsible.

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INTRODUCTION

The potato psyllid, Paratrioza cockerelli (Sulc.), has long been a major pest in potato growing areas because of the toxicogenic effect of the nymphal stage which produces psyllid yellows. With the possibility of a moratorium on insecticides, the need for alternate methods of control, such as biological control, becomes of prime importance. Although various writers have mentioned parasitism of the potato psyllid, little detailed work has been done on these parasites. Pletsch (1947) in a review of the literature on the potato psyllid gave a brief history and biology of one such parasite, Tetrastichus triozae Burks. From his observations and study, he concluded that the parasite should not be relied on as a factor in potato psyllid control. Dr. T. O. Thatcher and the author have observed high rates of parasitism in localized areas in Weld and Larimer counties in Colorado. This study was undertaken to determine some of the factors affecting the effectiveness of the parasite, T. triozae, in the potato growing areas of Weld and Morgan counties.

LITERATURE REVIEW

Although identification was not confirmed, there are indications in the literature of the association of T. triozae with the potato psyllid. Richards and Blood (1933), in reporting the 1928 potato psyllid outbreak in Utah, commented on the presence of parasites as a possible factor in psyllid control. Schaal (1938) mentioned parasitized nymphs in the Greeley, Colorado area when discussing the seasonal changes in potato psyllid populations. Romney (1939) also mentioned parasitism of the potato psyllid on uncultivated hosts in Arizona.

Burks (1943) reported collections of the parasites from Colorado, Kansas, New Mexico, Arizona, California, Idaho, Montana, and Washington. Jensen (1957) listed the following psyllid hosts from which the parasite had been reared: Artytaina minuta Crawf., Calophya californica Schw., C. nigrella Jensen, C. nigripennis Riley, C. triozomina Schw., Euphalerus vermiculosus Crawf., Pexopsylla cercocarpi Jensen, Trioza albifrons Crawf., T. beameri Tuthill, and the amelanchier psyllid (Trioza sp.).

Pletsch (1947) made the only extensive study on the biology of the parasite. He made some field observations and a laboratory study of the biology of the parasite in Billings, Montana. On August 28, 1939, he observed parasitized potato psyllid nymphs on

tomatoes. Later collections (September 26) showed a 23% parasitism at the Billings site. Although there was a heavy psyllid population, no parasitism was found in the surrounding area. In 1940 and 1941 only a few parasitized nymphs were found and they were all near the original location. These parasites were sent to the U. S. National Museum and were later described by Dr. B. D. Burks as Tetrastichus triozae.

Pletsch reared the parasites in the laboratory in 3 dram vials or in lamp chimney cages enclosing psyllid infested plants. The adults laid eggs only on fourth and fifth instar nymphs, which correlated with field observations. He found that psyllid nymphs were temporarily paralyzed and the eggs were laid on the ventral surface, usually between the coxae of the first and second or second and third pair of legs. Usually 1 egg was laid per nymph and in cases where more than 1 egg was laid only 1 larva developed.

Pletsch noted that parasite eggs hatched at room temperature in 1-2 days and the larvae fed externally for 6-8 days until the psyllid nymph died. The larva then glued the nymphal psyllid shell to the leaf and pupated within this shelter. As the pupa developed, the nymphal shell changed from a green hue to a rust-red color. Development was completed in 9-11 days, with the pupa changing from cream, to orange, to gray and finally to glossy black. The parasite emerged

by cutting through the top of the nymphal shell. The complete cycle lasted 16-19 days for the males and 17-20 days for the females.

Adults mated within 24 hours after emergence, with the matings lasting from 3-25 minutes. The shortest period from adult emergence to egg deposition was 2 days for mated females and 3 for unmated females. Longevity for 19 females was 5-14 days, with the average 9.63. Unmated females produced only males but mated females produced both males and females. Both mated and unmated females laid their eggs on fourth and fifth instar host nymphs. However, all wasps emerging from the fourth instar nymphs were males and those from the fifth instar were both males and females.

METHODS AND MATERIALS

Field observations on potato psyllid populations were made on potatoes, Solanum tuberosum L., in commercial fields and on matrimony vine, Lycium halimifolium Mill., in the potato growing areas of Weld and Morgan counties. These observations included population counts made in connection with the State Survey Program. Adult psyllid counts were made in commercial fields in the Fort Lupton, Gilcrest, La Salle, Greeley, Lucerne, Ault, and Prospect Valley areas of Weld county and in the Wiggins-Bijou Hill area of Morgan county. Psyllid (adult, nymph, and egg) counts were taken on Lycium from sites in Keensburg, Fort Lupton, Gilcrest, La Salle, and west of Greeley, These counts were used to show the psyllid population trend in the commercial fields as compared to that on the Lycium.

Adult counts were made by taking the total psyllid count and total number of sweeps with a standard 15-inch net for each observation day and converting them to number of psyllids per 100 sweeps. Nymph and egg counts were made by estimating the number of nymphs and eggs per leaf for each observation site. Counts were converted to number per 100 leaves for each observation day.

Field observations were made on the parasite to determine the time of appearance, abundance, and interactions with the host. Parasitized nymphs were collected from one site in Weld county during

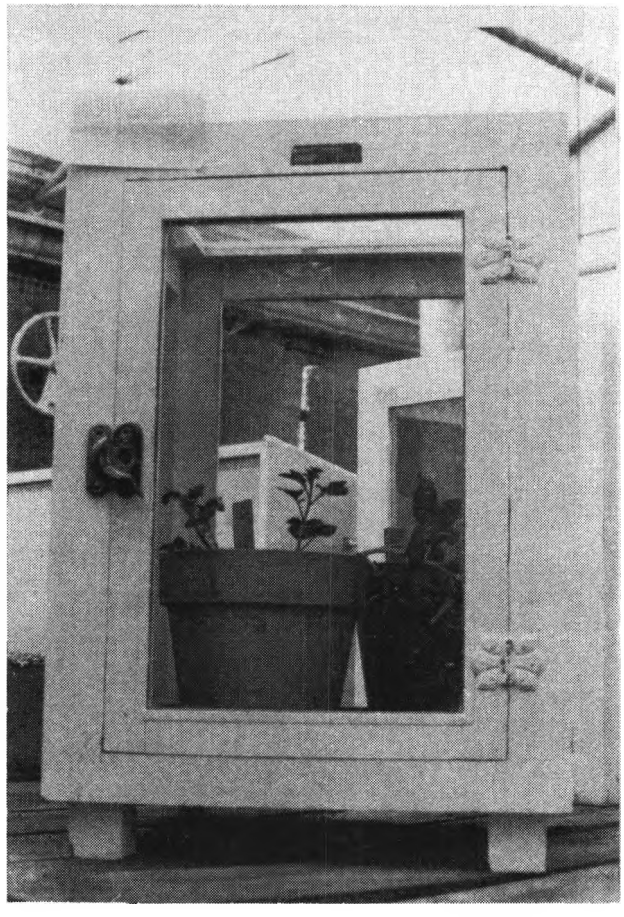
1969 to establish a parasite culture in the laboratory. Three parasites were sent to Dr. B. D. Burks of the U. S. National Museum for identification. The dead unemerged parasite pupae were examined for cause of mortality. During 1970, parasitized nymphs were collected at 3 sites in Weld county. The parasitized nymphs were collected and the parasites were allowed to emerge in petri dishes in the laboratory.

A potato psyllid culture was established in the Colorado State University Insectary. This culture was maintained in 18"x17"x24" wooden insect cages, with a glass paneled hinged door. The top and back of each cage had glass panels, while the sides had fine wire mesh (Figure 1).

Potatoes were used as the psyllid host. The varieties Norgold Russet and Russet Burbank were used because they were commonly grown varieties in Colorado and were most readily available. These potatoes were grown in 8-inch pots in organic soil. The seed potatoes were obtained from the Cogburn Potato Company in Eaton, Colorado. Fresh seed potatoes were obtained from the Potato Virus Laboratory during the winter months when they were unavailable on the market.

Changing and maintenance of the psyllid cultures were accomplished by 2 methods. When a completely new culture was desired, the cages were hosed down with water through the wire mesh and the water soaked contents removed. This prevented the escape of winged adults and facilitated the cleaning of the cages. A new culture was

Figure 1. Wooden cage used for the parasite and psyllid cultures.



started with adults and/or nymphs. Adults were added to the cages with an aspirator and nymphs were transferred with a camel hair brush. The brush method was used sparingly since a high mortality occurred when nymphs were transferred in this way. Nymphs were also added by draping an infested leaflet over a healthy plant and allowing the nymphs to transfer as the leaflet dried. This was a more efficient method, but it did not allow for establishment of exact populations and also presented problems when unwanted pests were present.

If continuation of the culture was desired, the plants were changed by removing the old plants carefully to prevent escape of the adults. In some cases, the old plant was draped over a new one or the adults remaining in the cage after removal of the infested plant were used to maintain the population. This method presented problems in maintaining pure cultures, especially if unwanted pests were present.

Parasite cultures were maintained in somewhat the same manner as the psyllid cultures. When the parasitized psyllid nymphs appeared on the leaves, they were put in petri dishes and the adult parasites were allowed to emerge. Adults were released in the psyllid cultures as needed in a manner similar to that described for the psyllids.

Parasite-psyllid cultures were maintained in petri dishes by putting the psyllid nymphs on fresh leaflets. The leaflets were kept

fresh by placing the petiole into a small stoppered vial through a small hole in the side. Because of the small size of the parasite and the flatness of the psyllid nymphs, some of the insects were able to escape between the lip and cover of the petri dish.

A series of laboratory experiments was set up to study the synchronization of the parasite and psyllid life cycles, mortality of the parasite pupae, parasitization by the parasite, the effect of predators on parasite mortality, and the cause of mortality. Experiments 1, 2, and 3 were conducted as general experiments to observe parasitization, parasite mortality, and parasite-psyllid synchronization. Experiment 4 was designed to study predators and disease as causes of mortality. Experiment 5 was set up to determine the search and find ability of the parasite, parasitization potential of the parasite, and to study synchronization of the parasite-psyllid cycles. The temperature and humidity in the greenhouse were recorded with a Bendix Hydrothermograph during all the experiments.

RESULTS AND DISCUSSION

Field Observations

Adult psyllid populations in commercial fields appeared in late May or early June, peaked, and declined. In 1968 and 1969, the populations peaked early in July and were negligible by the end of July (Tables 1 and 2). During 1970, adult psyllid counts peaked in mid-June and were at a low level by the end of June (Table 3). List (1939 a and b) indicated that this early season build-up followed by a decline when hot summer temperatures came was a normal trend for psyllid populations in Colorado. This trend was also reported in Arizona (Romney 1939), Texas (Janes 1936), Nebraska (Wallis 1940, 1946), and New Mexico (Jensen 1954).

Psyllid populations on Lycium during the 1969 and 1970 seasons developed throughout the summer (Tables 2 and 3). Adult psyllids appeared on the Lycium earlier than in the commercial fields and populations were higher throughout the season. This development throughout the summer was possibly due to the cooler environment created by the large shade trees at the study sites. The psyllid nymphs were abundant when the first parasitized nymphs were found (Tables 2 and 3). In 1968, psyllid populations declined rapidly in July on Lycium and in commercial fields (Table 1).

Table 1. Adult psyllid population development in commercial potato fields and psyllid and parasite population development on Lycium in 1968.

Date	Adults on Potato	Adults on Lycium	Nymphs on Lycium	Eggs on Lycium	Parasites on Lycium
May 29	0[a]	19[a]	0[b]	0[b]	
June 30	5	16	0	12	
June 4	8	19	0	500	
June 7	0	32	0	600	
June 11	7	37	0	500	
June 18	4	46	500	1000	
June 27	2	60	200		
July 2	10	100	100		
July 16	2				
July 18	5	0	0	0	
July 25	0	0	100		0[c]
July 30	0				
Aug. 8	0				

[a] Adult counts per 100 net sweeps.

[b] Nymph and egg counts per 100 leaves.

[c] Parasites did not appear on Lycium before July 25 and no observations were made after that date.

Table 2. Adult psyllid population development in commercial potato fields and psyllid and parasite population development on Lycium in 1969.

Date	Adults on Potato	Adults on Lycium	Nymphs on Lycium	Eggs on Lycium	Parasites on Lycium
May 1	0[a]	10[a]	0[b]	400[b]	
May 24	9	20	0	100	
June 4	0	20	0	100	
June 12	0				
June 25	2		200		
June 30	1				
July 7	18				
July 9	20				
July 14					0[c]
July 24	5	100	500		
July 25					13
July 28	5				
July 29	7				
Aug. 5	0	150	800		12
Aug. 8	0				
Aug. 14	0				
Aug. 18		200	200		76
Aug. 21	0				
Sept. 3	0				
Sept. 9	0				
Sept. 19	0		0	200	0
Sept. 26	0	8	12	1000	0
Oct. 2	0		20	1050	0

[a] Adult counts per 100 net sweeps.

[b] Nymph and egg counts per 100 leaves.

[c] Parasite counts are the total number of rust-red parasitized psyllid nymphs present at the Gilcrest site on that date (all plants at the site were examined thoroughly).

Table 3. Adult psyllid population development in commercial potato fields and psyllid and parasite population development on Lycium in 1970.

Date	Adult on Potato	Adult on Lycium	Nymphs on Lycium	Eggs on Lycium	Parasites on Lycium
May 14	0[a]	0[a]	0[b]	0[b]	
May 21	0	72	0	750	
May 28	0				
June 1	0	120	0	400	
June 3	0	100			
June 5	7				
June 15	10				
June 16	2				
June 19	0				
June 23	2	46	400	1000	
June 29	1	200	500	500	
June 30	0				0[c]
July 10	0		650		26
July 14	0				
July 17	0				
July 22	0				
July 28	0		500		71
Aug. 4	0				
Aug. 14	0	100	200		27
Aug. 19	0	125	50		6
Aug. 28	0	14	8	28	0

[a] Adult counts per 100 net sweeps.

[b] Nymph and egg counts per 100 leaves.

[c] Parasite counts are the total number of rust-red parasitized psyllid nymphs present at the Gilcrest, La Salle, and Greeley sites on that date (all plants at the sites were examined thoroughly).

In 1969, rust-red parasitized psyllid nymphs (first readily detectable sign of parasitism) were first observed at the Gilcrest site on July 25 (Table 2). According to Pletsch, this indicated that the eggs would have had to have been laid around July 15. Parasitism reached its peak in the middle of August (Table 2). Toward the end of August maturation caused the Lycium leaves to drop. In September, new leaves appeared and psyllid populations developed again, but no parasitized nymphs were found.

In 1970, rust-red parasitized nymphs appeared at the Gilcrest, La Salle, and Greeley sites on July 10, two weeks earlier than in 1969 (Table 3). The eggs would have had to have been laid around July 1 (Pletsch 1947). As in 1969, parasitism reached a peak within a month after the eggs were laid and then declined (Tables 2 and 3). Adult parasites were present at the end of August, but no parasitized nymphs were found.

The parasites appeared in 1969 and 1970 after the spring psyllid infestation had declined in the commercial fields (Tables 2 and 3). By the time the fall psyllid populations had developed, the parasite populations had declined. Shibuya and Maebara (1953) reported from Japan that the pod gall midge parasite, Tetrastichus sayatamabae, also had a late appearance and rapid decline in August. Thus, this trend is known among other species of Tetrastichus. This lack of synchronization of the parasite with the heavy psyllid

infestations may be one reason for the ineffectiveness of the parasite as a biological control agent on the potato psyllid in Weld and Morgan counties.

Parasite pupal mortality was high in the samples collected in the field (Table 4). In 1969, mortality ranged from 46-100% and averaged 88.1%. During 1970, mortality ranged from 38-78% and averaged 65.6%. Only 25% of the parasites obtained in the field emerged. This high mortality may also be a factor affecting the effectiveness of the parasite.

The cause of the mortality was not ascertained. Some of the dead pupae had the symptoms described by DeBach (1965) for viral and bacterial diseases of insects. Others appeared as if the internal fluids had been extracted. Anthocorids, Nabids, Reduviids, Phymatids, and Chrysopa larvae were observed as host associates of the psyllid and parasite at the Lycium sites An immature Anthocorid, Orius sp., was observed probing at a parasitized psyllid nymphal shell in one case. It was speculated that disease and/or predators may be factors in the high field mortality.

Parasite-Potato Psyllid Interactions

Two experiments were conducted in the greenhouse to study the synchronization between the parasite and psyllid life cycles, parasitization by the parasite, parasite mortality, and the effect of the predators on the parasite. Experiment 1 was done during

Table 4. Mortality of parasite pupae present under rust-red parasitized psyllid nymphs collected in Weld county in 1969 and 1970.

Date	Parasite pupae			
	Total[a]	Emerged	Dead[b]	% Mortality
July 25	13	7	6	46.2
Aug. 5	12	0	12	100.0
Aug. 18	76	5	71	93.4
Total for 1969	101	12	89	88.1
July 10	24	15	9	37.5
July 23	71	16	55	77.5
Aug. 14	27	11	16	59.3
Aug. 19	6	2	4	66.7
Total for 1970	128	44	84	65.6
Grand Total	229	56	173	75.5

[a] Total number of rust-red parasitized psyllid nymphs collected on that date.

[b] Cause of mortality was not ascertained, but some of the pupae had the symptoms described by DeBach (1965) for viral and bacterial disease; while others appeared as if the internal fluids had been extracted.

September, October, and part of November and consisted of 5 cultures in which general observations were made. Experiment 2 was carried out during November and December and consisted of 5 cultures, 4 with predators and 1 without.

Experiment 1 was designed to make a general study of parasite-psyllid synchronization, parasitization, and parasite mortality. In each culture, a parasite-psyllid population was observed from an initial number (different in each culture), through subsequent development of the various stages of psyllid and parasite, to a final population. Data recorded for each culture included: number in initial population, number and time of appearance of the various stages of the psyllid, number and appearance of the rust-red parasitized psyllid nymphs, and the number and appearance of the emerged adult parasites. The temperature and humidity in the greenhouse were recorded with a Bendix Hydrothermograph during the experiment.

The parasite-psyllid population in each culture was allowed to develop on two consecutive potato plants. When psyllid yellows became severe on the first plant, it was cut and draped over a new plant. After allowing sufficient time for the psyllid nymphs to transfer to the new plant, the first plant was placed in a gallon jar and the final population was recorded. When the damage on the second plant became severe, this plant was cut, placed in a gallon jar, and the final

population recorded. Sufficient time was allowed for parasite emergence in the gallon jars and those parasites from the first plant were released back into the culture.

Final information recorded for each plant included: number of emerged parasites, number and stage of development of living parasite pupae, number and stage of development of dead parasite pupae, number of psyllid nymphs, and the number of psyllid adults. The number of emerged parasites was obtained by counting the number of parasitized psyllid nymphs with parasite emergence holes. Parasite pupal development was classified as early (when the pupae were in the cream to orange color phase) and late (when the pupae were in the gray to black color phase). Percent parasitization and percent mortality were based on the total populations recorded in each culture.

Parasite development time agreed with that given by Pletsch (1947). The egg to pupae phase took 8-10 days and the pupae to adult phase took 8-10 days. Psyllid development took 3-4 weeks, so that once the parasite was synchronized with the suitable psyllid stage, parasite emergence was staggered enough to maintain synchronization. No clear cut relationship existed in the 5 cultures between the ratios of the number of parasites originally released (ranged from 10-199) and the number of parasite offspring produced (Table 5). But it appeared that there may have been a relationship between the

Table 5. Psyllid parasitism and parasite mortality in relation to orginal populations released.

	Cultures					
	1	2	3	4	5	Total
Original population released						
Adult psyllids	1	20	50	0	4	75
Psyllid nymphs	63	65	81	100	10	319
Parasites	10	25	58	72	199	364
Final population recorded						
Adult psyllids	164	960	459	133	40	1756
Psyllid nymphs	2130	1096	5124	6912	3743	19005
Parasites	970	559	2934	3619	2995	11077
Total parasite mortality	155	92	234	399	580	1460
Percent mortality	16.0	16.5	8.0	11.0	19.3	13.2
Ratio of original parasites released to parasite offspring produced	1:97	1:22	1:50	1:50	1:15	
Length of culture in days[a]	41	38	38	57	42	
Number of hours above 70°F.[b]	895	810	810	1038	393	3946

[a]The length of the cultures varied because the length of time for psyllid yellows to kill the plants varied. Populations were carried through 2 consecutive potato plants.

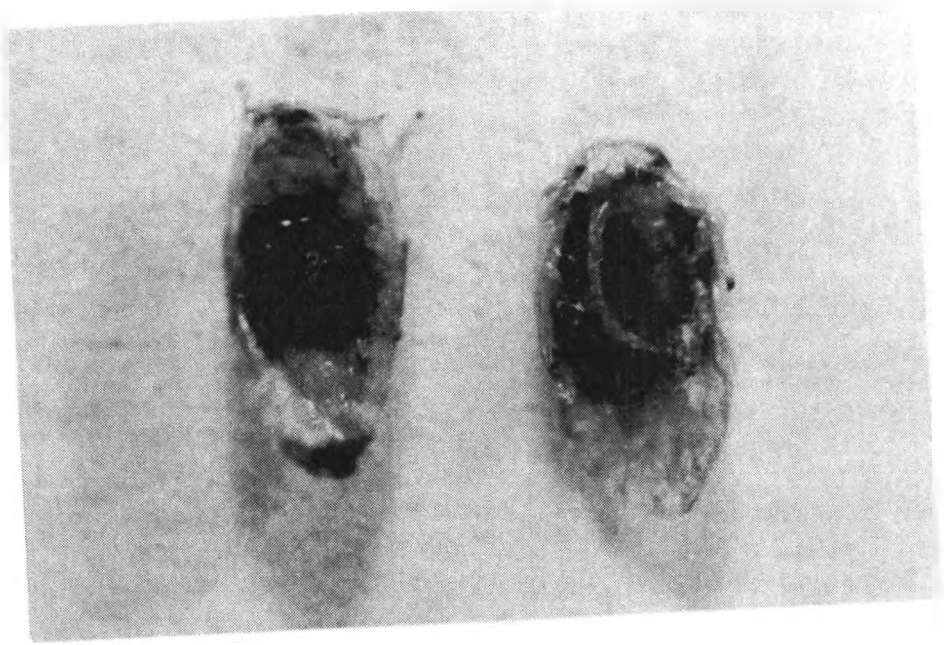
[b]The hours above 70°F. varied because the cultures were not run simultaneously, although they were run concurrently during September-November 1970.

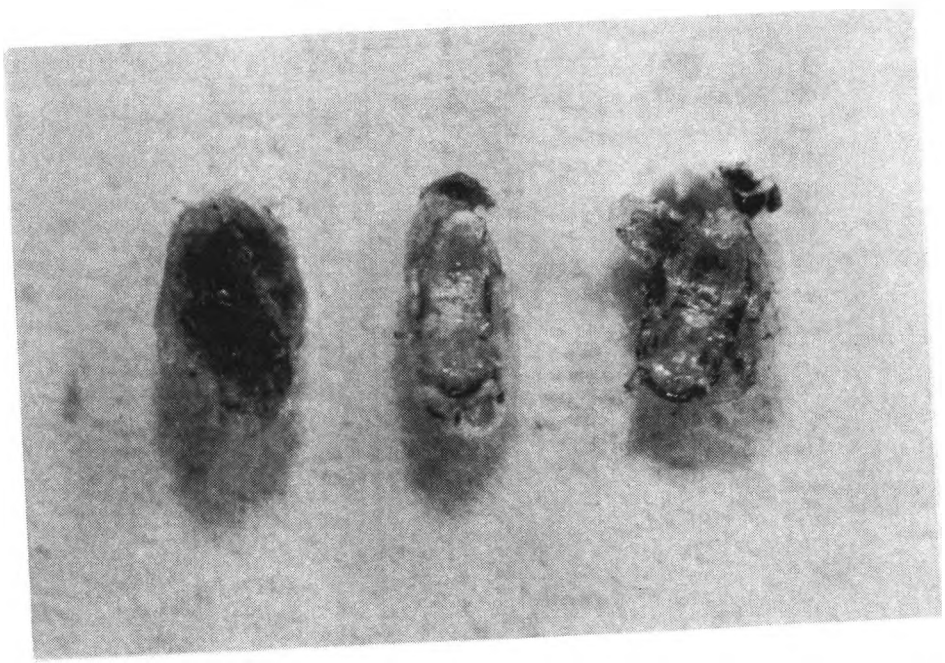
number of parasites originally released and percent parasitization, with the larger releases producing a higher percent parasitization (Table 5).

Parasitization in the 5 cultures ranged from 27-79% and averaged 53.3%, while mortality ranged from 8-19% and averaged 13.2% (Table 5). Although parasitism was high in the culture, the parasite was unable to control the psyllid nymphal populations enough to prevent plant destruction by psyllid yellows. This may have been due to the highly prolific nature of the psyllid, especially under the confined conditions of the cages, and the fact that the parasite only attacked the fourth and fifth instar nymphs. This attacking of the fourth and fifth instar nymphs was noted by Pletsch (1947) and was found to be true in this study also. Parasite mortality in the cultures was not as high as that in the field although the dead parasite pupae resembled those found in the field (Figure 2).

Since potential predators had been observed in the field associated with the psyllids and parasites, experiment 2 was designed to study the general effect of predators on the parasite-psyllid development. The cultures were handled similarly to those in experiment 1, except for the presence of predators and the treatment given the second plant. Plant 2 in the cultures with predators, after being placed in the gallon jars, was subjected to heat to hasten the drying of the plant. The gallon jars were placed simultaneously on a radiator for 12 hours. This was done because it was easier to

Figure 2. Some examples of the parasite pupal mortality from experiment 2. Dead parasite pupae similar to these were found both in the field populations and in the laboratory cultures. (Taken January 21, 1971 with a Minolta SR-1 on Panatomic X 135 through a cycloptic microscope, 600x and 300x.)





tally the final populations on dryer plants. Percent parasitism and percent parasite pupal mortality were not appreciably different between plant 1 (which was not placed on a radiator to dry) and plant 2, so the final populations were treated as in experiment 1.

The predators used in each of the cultures were as follows: culture 1-2 Orius tristicolor, culture 2-1 Nabis sp., 2 Orius insidiosus; culture 3-1 Orius insidiosus; and culture 4-1 Nabis sp., 1 Orius insidiosus. These predators were used because they were most numerous at the Lycium sites and were available when the experiment was carried out.

Parasitism in the cultures with predators ranged from 23-47% and averaged 28.6%. Mortality ranged from 13-52% and averaged 26.5% (Table 6). There was no significant difference in percent mortality or percent parasitism between the cultures with the predators and the control indicating that the predators were not significant factors in parasitism nor parasite mortality.

During experiments 1 and 2, it was observed that the parasite would sting the psyllid nymphs but not lay eggs and that there were many rust-red dead psyllid nymphs without parasite eggs, larvae or pupae under them. Burnett (1967) found that Encarsia formosa Gahan would sting its host without laying eggs, which was a controlling factor in the parasite-host population fluctuations. Experiment 3 was conducted to see if the rust-red psyllid nymphal shells without

Table 6. The effect of predators on psyllid parasitism and parasite mortality.

	Cultures with predators[a]					Culture without predators[b]
	1	2	3	4	Total	Control
Original population released						
Adult psyllids	80	50	100	100	330	100
Psyllid nymphs	100	30	100	100	330	100
Parasites	45	30	30	5	110	5
Final population recorded						
Adult psyllids	1350	20	117	873	2360	6
Psyllid nymphs	3729	2652	3328	1454	11154	2900
Parasites	1149	1243	930	539	3861	694
Total parasite mortality	266	163	485	110	1024	227
Percent mortality	23.2	13.1	52.2	20.4	26.5	32.7
Percent parasitization	22.6	46.5	27.0	23.3	28.6	23.9
Length of culture in days[c]	37	42	39	42		35
Number of hours above 70°F.[d]	872	987	915	987	3761	840

[a] Predators used: culture 1-2 Orius tristicolor; culture 2-1 Nabis sp., 2 Orius insidiosus; culture 3-1 Orius insidiosus; culture 4-1 Nabis sp., 1 Orius insidiosus.

[b] No significant difference between cultures with predators and without, using the Chi-Square Test.

[c] The length of the cultures varied because the length of time for psyllid yellows to kill the plants varied.

[d] The hours above 70°F. varied because the cultures were not run simultaneously, although they were run concurrently during November-December 1970.

parasite eggs, larvae or pupae under them were caused by the stinging of the parasite or by nymphal mortality due to other causes.

The experiment consisted of 15 clip-on cages placed on the surface of the potato leaves. In each of 10 cages, 10 fourth or fifth instar psyllid nymphs and 1 adult parasite were confined. In the remaining 5 cages, 10 fourth and fifth instar psyllid nymphs were confined without the parasite. These served as the control for the experiment. The psyllid nymphs were examined for mortality after 2 weeks. Results were categorized as rust-red psyllid nymphal shell without parasites, rust-red nymphal shell with parasites, emerged parasites, and emerged adult psyllids.

In the 10 cages with parasites, nymphal mortality ranged from 10-100% and parasitism 0-50%. This wide variation may have been due to the premature escape of the parasite from some of the cages. In all of the cages from which the parasite did not escape, there was 100% psyllid nymph mortality. There was 100% psyllid adult emergence in the control cages. Mortality data are shown in Table 7. In the cages with parasites, both the dead psyllid nymphal shells with the parasite pupae and without were rust-red. The absence of any rust-red psyllid nymphal shells in the checks indicated that all mortality observed in the cages with parasites was caused by the parasite. Since no parasite eggs or early larvae were found under many of the rust-red nymphal shells, it appeared that either the parasite did sting the nymph without laying eggs or the eggs were overlooked.

Table 7. Parasitism by T. triozae and mortality of psyllid nymphs in relation to the occurrence of rust-red nymphal shells.

	Means			
	No. of rust-red nymphal shells[c]	No. of rust-red parasitized nymphs[d]	No. of adult parasites emerged	No. of adult psyllids emerged
Cages with parasites[a]	4.1	1.4	0.9	4.5
Cages without parasites[b]	0.0	0.0	0.0	10.0

[a] Mean based on 10 clip-on cages each containing 10 psyllid nymphs confined with 1 adult parasite.

[b] Mean based on 5 clip-on cages each containing 10 psyllid nymphs which were used as the control.

[c] Without parasite egg, larvae, or pupae underneath.

[d] With parasite pupae underneath.

As in Burnett's study, this stinging without laying eggs could be a significant factor in the T. triozae-potato psyllid interaction. Very few of these rust-red psyllid nymphal shells without parasites were found in the field populations; possibly because in the field the shells were more readily dislodged from the leaf surface.

Predators and Disease as Causes of Mortality

A series of experiments was designed to study the relationship of predators and disease to parasite pupal mortality in more detail. The insidious flower bug, Orius insidiosus (Say), was used because it was available when the experiment was carried out and because an immature Orius sp. was observed probing at a parasitized psyllid nymph in the field. First, the flower bugs were confined in petri dishes with live and parasitized psyllid nymphs and the feeding response was observed. Next, the flower bug was released in parasite-psyllid cultures and the effect on parasite pupal mortality was studied. Finally, a number of parasite pupae were taken to the U.S.D.A. Bee Disease Laboratory in Laramie, Wyoming, and examined by Dr. W. T. Wilson for bacteria and inclusion bodies with a phase contrast microscope.

A series of petri dishes was set up in which the flower bug could be observed feeding on parasite pupae and psyllid nymphs. First, an immature and an adult flower bug were confined with 2 healthy fifth instar psyllid nymphs and 2 parasitized nymphs on a potato leaflet.

Observations were made for 5 hours. Both flower bugs fed readily on plant juices and the psyllid nymphs. The immature bug fed for 37 minutes on 1 nymph and the adult probed on the other. Both psyllid nymphs were dead when examined. Although the flower bugs walked over the parasitized nymphs and hesitated several times, no probing nor feeding was done through the protecting nymphal shells.

Next, to determine if the flower bugs would feed through the protecting shell or on naked parasite pupae when plant juices were not available, the bugs were confined with 5 parasite pupae for 5 hours. Four of the pupae were protected by the psyllid nymphal shell and 1 was exposed. The immature flower bug fed readily on the exposed parasite pupa for 50 minutes. At one point the adult bug took the parasite pupa away from the immature flower bug. Although the adult dropped the pupa and did not appear to feed on it, this action indicated that the adult may also feed on the unprotected pupae. The pupal remains, after the immature flower bug had finished feeding, resembled the dead pupae observed in the field and in the laboratory cultures (Figure 2). Neither of the flower bugs fed on the protected pupae although they passed over them several times.

Finally, to determine if the flower bugs would feed through the protecting psyllid nymphal shell under starvation conditions, the immature flower bug, after being starved for 5 hours, was confined overnight with 3 protected parasite pupae in a small cage. The immature bug did not feed while the pupae were protected, but

readily fed on the parasite pupae when they were exposed mechanically the next morning. It appears that the insidious flower bug will feed on potato plant juices, psyllid nymphs, and T. Triozae pupae if it can get to them.

During these studies a Nabid, Nabis sp., was also confined on a leaf surface by a clip-on cage for 2 days with 3 parasitized psyllid nymphs, 4 healthy fifth instar psyllid nymphs, and 2 adult psyllids. The Nabid died without feeding.

A final experiment was designed to determine if the flower bug was a significant factor in parasite pupal mortality. It consisted of 6 insect cultures, 3 with psyllids, parasites, and predators and 3 with psyllids and parasites, but no predators. All the host plants were planted on the same date and were approximately the same height and condition at the start of the experiment. A leaflet with approximately 50 second and third instar psyllid nymphs was placed on each healthy plant and the insects were allowed to transfer. Second and third instar nymphs were used to insure that no previously parasitized psyllid nymphs were present. Ten adult parasites were added to each culture after the nymphs had transferred. Eleven days later 2 Orius insidiosus were introduced into 3 of the cultures. The experiment was terminated after 17 days and the following information was recorded: number of emerged parasites, number of dead parasite pupae, number of adult psyllids, and the number of psyllid nymphs.

Mortality in the cultures with predators was 13.0% and the percent parasitism 67.7 (Table 8). This was not significantly different from the check which had 11.3% mortality and 65.7% parasitism (Table 8). Thus, flower bug predation was not a significant factor in the parasite pupal mortality and did not affect parasitization. Note the difference in psyllid nymphal mortality and adult emergence between the cultures with predators and those without indicating possibly that the flower bug was feeding on the psyllid nymphs (Table 8).

Dr. W. T. Wilson of the U.S.D.A. Bee Disease Laboratory examined about half of the dead parasite pupae from this experiment. He found some cocci bacteria and possibly some polyhedral inclusion bodies in a few of the pupae. He felt that the small number of disease organisms present indicated that disease was not a significant factor in the parasite pupal mortality.

Searching and Finding Ability of the Parasite

It was observed during experiments 1 and 2 that the adult parasites were weak fliers, making only short, avoidance flights when disturbed. It was speculated that this might be a factor in the effectiveness of the parasite. A series of experiments was thus set up to study the search and find ability of the parasite. Three preliminary releases and observation and 1 detailed experiment were conducted.

Table 8. The effect of the predator, Orius insidiosus, on the mortality of Tetrastichus triozae and the efficiency of the parasite as a control for the potato psyllid.

	Means[a]	
	Cultures with predators[b]	Cultures without predators[c]
Total parasite population[d]	47.7	52.3
Dead parasites	6.7	5.3
Total psyllid population[d]	23.3	25.6
Emerged psyllid adults	8.3	17.3
Dead psyllid nymphs	15.0	8.3
% Mortality	13.0	11.3
% Parasitization	67.7	65.7

[a] Represents the mean of 3 cultures.

[b] Two Orius insidiosus per culture.

[c] No significant difference between the cultures with the predators and without (Chi-Square Test).

[d] Initial population was approximately 50 psyllid nymphs and 10 adult parasites.

The preliminary releases were made from various points in the greenhouse and observations were made on a test plant to see if and when the parasites arrived at the plant. On October 28, 2 adult psyllids, psyllid eggs, and 1 adult parasite which escaped from the near-by cultures were observed on the test plant. On November 13, 75 parasite adults and 25 adult psyllids were released 30 feet from the test plant which at that time was infested by psyllid nymphs. Two days later, none of the parasites could be found on the test plant nor anywhere in the greenhouse, including in the numerous spider webs in the area of the release.

A second release of 35 parasites and 15 psyllids was then made at a point 12 feet from the test plant. The next day 1 parasite and 2 adult psyllids which apparently originated at the release point were found on the test plant. Twenty-five parasites and 10 psyllids were again released from 12 feet away. After 10 days, 5 parasites were observed on the test plant. No additional parasites were found 30 days later when the test plant was examined and the parasitization and mortality was tallied. Fifty-three precent of the psyllid nymphs were parasitized and the parasite pupal mortality was 5%.

Because of the possibility of contamination of the test plant from the near-by parasite-psyllid cultures, the releases were repeated later in another greenhouse. One-hundred parasites and 100 adult psyllids were released 12 feet from a test plant on November 15.

Two days later, 15 parasites and 5 adult psyllids were released 7 feet from the test plant. No parasites nor psyllids were found on the test plant after 13 days. It appeared that distances longer than 7 feet were too great for the parasite to move easily and that the distance between host plants may be a factor in parasite distribution and effectiveness.

Experiment 5 was designed to study the search and find ability and the effectiveness of the parasite as influenced by the distance between host plants and the size of the psyllid nymph population. The experiment was set up in the greenhouse with pots of potato plants arranged in the pattern indicated in Figure 3. The pattern was designed to determine if the distance between plants or the number of psyllid nymphs present on the plants would interfere with the parasite distribution. On December 22, approximately 100 adult parasites and 750 adult psyllids were released from the center of the pattern. The release was made by opening the door of a cage with a parasite-psyllid culture in which the plant was dying and allowing the adults to disperse. The insects left the cage within 2 days and the cage was removed. Various numbers of second and third instar psyllid nymphs were transferred to various plants in the pattern 2 days previous to the release of the parasites (Figure 3).

Parasite and psyllid population development and counts were recorded periodically for each plant. The populations were divided into 2 categories: original population and developing population. The original population included the original nymphs and the parasite

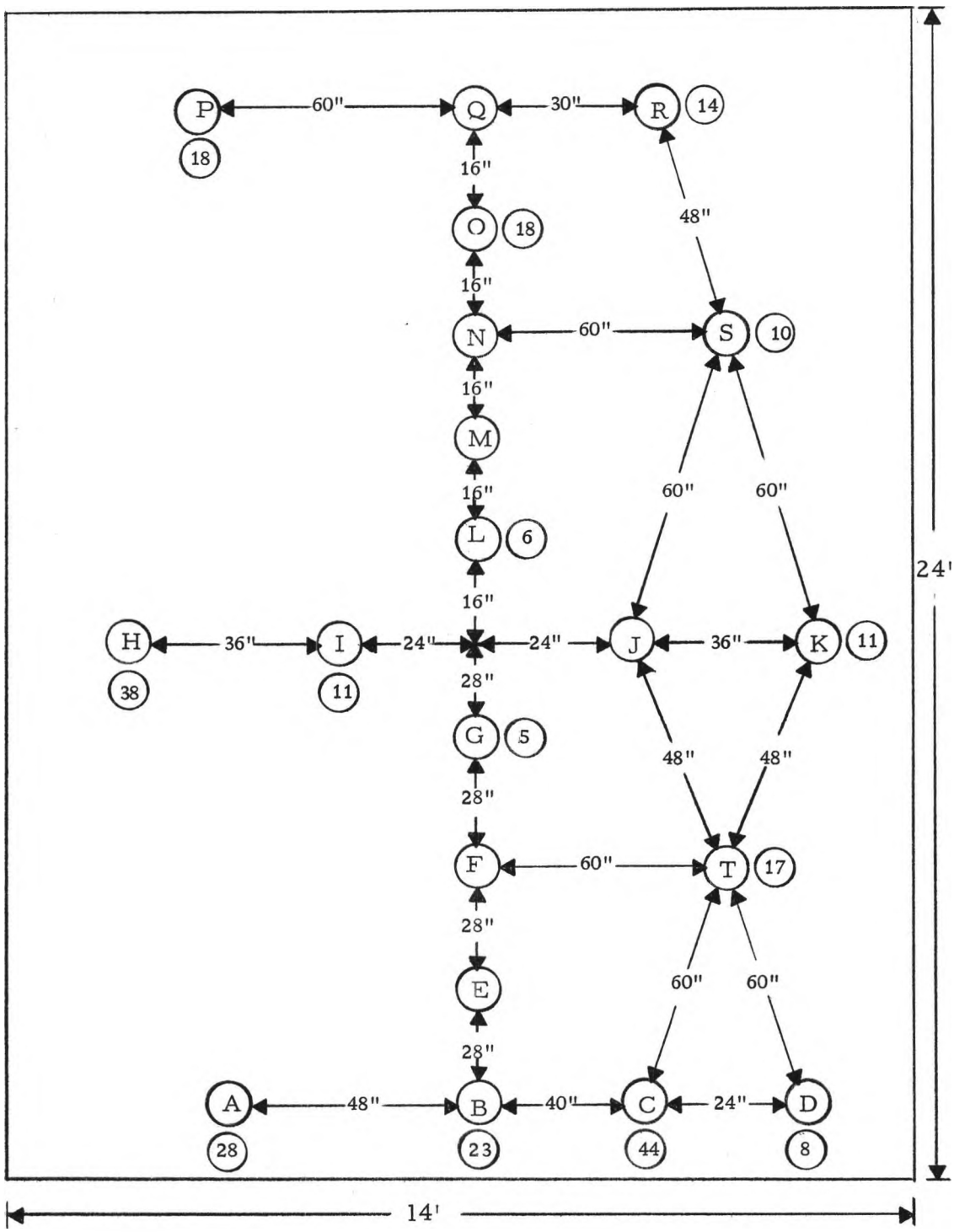


Figure 3. Distribution of potted plants used in the Search and Find Experiment. Distance between pots is shown by the numbers within the arrows. The original number of nymphs and the location of the pots where they were released is indicated by the circled numbers.

population which parasitized the nymphs. Data were recorded on the development of the original nymphs, the time of appearance and number of parasitized nymphs, and the number of emerged parasites. The developing population included the original adult psyllids, eggs laid by the original adults, and the subsequent development of the nymphs. Number and time of appearance of the various psyllid nymphal instars were recorded. This population was used to further study the synchronization between the parasite and psyllid.

On January 5, the experiment was terminated. The plants were cut, put in a gallon jar, and the populations counted. Information recorded was number of eggs, early instar psyllid nymphs (first to third), fourth instar nymphs, fifth instar nymphs, adult psyllids, parasitized nymphs, and emerged parasites. The unemerged parasites were cultured in petri dishes and the number emerging were recorded each day.

Among the original nymphs, development to adults, parasitization, or mortality had occurred within a week on all plants on which they were present. Parasitized nymphs appeared throughout the experiment on December 30 and 31. Assuming 8-10 days for parasite development from egg to pupa, parasitization must have occurred within the first few days after the parasites were released. Parasites emerged in the petri dishes from January 5-18, with the majority emerging on the tenth.

Adult psyllid distribution was as expected. The center plants were heavily infested within 1 day and all the plants were infested by December 29. Eggs had appeared on all plants by December 26. By the time the experiment was terminated, all stages of psyllid nymphs were present with the majority being early instars (Table 9). When numbers were too high to count, they were recorded as "numerous" (over 3000).

Parasitization of the original nymphal population ranged from 13-100% and averaged 74.7% overall. Seventy-nine and 2-tenth percent of the parasites emerged (Table 10). Immature Orius sp. were found on plants G and H. Only 7 nymphs in the population developing from the released psyllid adults were parasitized and these were on the center plants where suitable nymphal stages (fourth and fifth instars) had appeared earliest. Pletsch (1947) indicated adult parasite longevity in the greenhouse as 5-14 days. It was speculated that the lack of parasitism in the developing population was due to the decline of effectiveness of the original adult parasites, and the lack of an effective number of emerging parasites from the original psyllid nymph population. Subsequent emergence of the parasites indicated that synchronization between the psyllids and the parasites would probably have occurred had the experiment been continued.

From these experiments, it appears that the distance between plants did have an effect on the distribution of the parasite as shown

Table 9. Psyllid populations resulting from the release of adults[a] in the center of a pattern of potato plants.[b]

Plant	Egg Count	1st to 3rd Instar Nymphs	4th Instar Nymphs	5th Instar Nymphs
A	71	87	0	0
B	158	68	0	0
C	465	173	1	10
D	2766	1586	23	0
E	613	94	13	1
F	c	339	193	100
G	620		811	485
H		125	2	1
I			152	160
J			437	413
K			168	143
L			592	414
M			778	472
N			176	45
O			211	102
P	20	7	3	0
Q	227	42	25	14
R	1016	82	37	35
S			148	102
T	____	____	246	245
Total	5956	2603	4016	2742

[a] Seven-hundred and fifty adult potato psyllids were released.

[b] The potato plants were placed in a set pattern with distances varying from 16-60 inches between the pots.

[c] Numbers not given were estimated as over 3000.

Table 10. The movement of T. triozae[a] from a release point as measured by the parasitism of psyllid nymphs present on potato plants set in a specific pattern.[b]

Plant	No. of original psyllid nymphs	No. of nymphs parasitized	Total parasite mortality	Percent parasite mortality	Percent psyllid parasitization
A	28	22	4	17.2	78.6
B	23	20	4	20.0	87.0
C	44	41	10	24.4	93.2
D	8	1	0	0.0	12.5
G	5	5	1	20.0	100.0
H	38	36	5	13.9	94.7
I	11	6	1	16.7	54.5
K	11	3	0	0.0	27.3
L	6	4	0	0.0	66.7
O	18	13	4	30.8	72.2
P	18	12	6	50.0	66.7
R	14	9	2	22.2	64.3
S	10	4	0	0.0	40.0
T	17	16	3	18.8	94.1
Total	257	192	40	20.8	74.7

[a] One hundred parasites were released.

[b] The potato plants were placed in a set pattern with distances varying from 16-60 inches between the pots, with a specific number of nymphs on each plant (Plants E, F, J, M, N, and Q did not have any nymphs on them).

by the parasitization throughout the experiment within a short time after the release. The high percent parasitism on the small psyllid nymphal population indicated that the parasite had a very efficient search and find capacity.

General Discussion

This study indicated that the late appearance and rapid decline of parasite populations, pupal mortality, and flight capacity were all factors in the effectiveness of the parasite, T. triozae, as a control agent of the potato psyllid. The fact that the parasite appeared after the spring psyllid infestation reached a peak and declined before the fall psyllid infestation built up makes its value as an economic control agent highly questionable. This, coupled with the fact that it attacks only fourth and fifth instar psyllid nymphs and thus will not prevent potato plant destruction by psyllid yellows even under confined conditions, makes mass release in commercial areas economically unfeasible. However, observation in the cultures and in the open greenhouse regarding the control of the parasite leads the author to conclude that the possibility of mass releases in the overwintering areas of the psyllid in Arizona and Texas may be valuable and should be investigated.

Flight capacity is probably the main reason for the localized effect of the parasite. The parasite did not appear to be able to get to the test plants when released at distances of 30, 12, and 7 feet;

yet distribution was rapid when host plants were at distances of 5 feet or less from the point of release. This suggests that distribution may be by short flights from host plant to host plant. However, there is one other possibility for the lack of distribution in the preliminary releases, although evidence is circumstantial and highly questionable. It is possible that the quantity of light in the greenhouse was causing the parasites to go into hibernation. This supposition is based on the decrease in parasitism between experiment 1 and 2 and the increase during 4 and 5.

During experiment 1 and 2, florescent lights were used to supplement the natural light in the greenhouse. The parasitization rate during experiment 2 was half that in experiment 1 (Tables 5 and 6). The preliminary releases were made while experiment 2 was in progress. Westinghouse plant-gro florescent lights were added to the greenhouse during experiments 4 and 5 to compensate for the waning natural light. Parasitism in both experiments 4 and 5 was high compared to that in experiments 1 and 2 (Tables 5, 6, 8, and 10). The florescent lights were used progressively more during experiment 2 as the amount of natural light decreased. This decreased quantity of light may relate to the difference in parasitism between experiments 1 and 2; and the sudden increase in experiments 4 and 5 when the plant-gro lights were added. Although there are other variables that could have caused the variation in parasitism

between the experiments, the author suggests that the effect of light quantity on parasitization should be investigated further.

Parasite pupal mortality was high both in the laboratory and in the field. Laboratory mortality was as high as field mortality in only one instance. Experiment 4 showed that predators and disease, although possibly causing some mortality, were not important factors in the overall picture. The major cause of the mortality was not ascertained, but temperature acting on the moisture in the micro-environment of the pupae may possibly be an important factor.

In instances where parasite mortality was recorded on plants which still retained a high amount of moisture, mortality was always low, sometimes as low as 3-5%. The results of the heat treatment during experiment 2 also indicated the importance of moisture content of the plant. Plant 2 in the 4 cultures with predators were subjected simultaneously to 12 hours on the radiator. The plant from culture 3 had been cut 2 days previous to the other 3. Just before being put on the radiator, a sample was taken from plant 2 in culture 3 and all the pupae examined were alive. After the heat treatment, all pupae on this plant were dead. The mortality on the other 3 plants was not appreciably affected. This would seem to indicate that the extra drying that this plant underwent previous to the heat treatment was a factor in the mortality.

Also, the temperature was higher in experiments 2-5 on the basis of hours above 70°F. Experiment 1 averaged 18 hours per day

above 70°F and the rest averaged 23 hours above 70°F. When the difference in temperature and humidity between experiment 1 and the remaining experiments are considered, there is sufficient difference to say that the variation in mortality could have been caused by the affect of the temperature on the microenvironment around the parasite. The evidence to support this is extremely poor and the author suggests this should be investigated further under conditions where more control is available.

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