Environmental Entomology Biological Control

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The potential of <u>Myzus lythri</u> (Homoptera:Aphididae) to influence the growth and development of <u>Lythrum salicaria</u>

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Abstract

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The host-alternating life cycle of <u>Myzus lythri</u>, an aphid of european origin that feeds on purple loosestrife (<u>Lythrum salicaria</u>), is detailed. The complex life cycle and low availability of primary host plants are hypothesized as limiting early season development of aphid populations on purple loosestrife. Experiments were undertaken in 1993 to determine if large poplations of this aphid could influence the growth and development of purple loosestrife. Two year plants each inoculated with five aphids showed significantly lower root and shoot weight after a seven week period as compared to matched controls. Over half of twenty three-month-old seedlings inoculated with two or five <u>M. lythri</u> were dead or clearly dying eleven weeks post inoculation. Mass rearing for early spring release and planting primary hosts are possible methods to augment the potential of this aphid to function as a biological control agent for purple loosestrife.

In a recent article Malecki et al. (1993) describe the biological control program for purple loosestrife in North America. They document, 1) the suitability of <u>Lythrum salicaria</u> as a target organism for biological control, 2) the research effort that has been expended in its native range, 3) the tests undertaken to ensure the host specificity of the phytophagous beetles to be released as control agents and 4) propogation and release strategies. The original selection of potential biological control agents was based on surveys in Europe (Batra et al. 1986). In the literature discussing potential biological control agents for purple loosestrife there is no reference to <u>Myzus lythri</u>, a European aphid now found throughout much of United States and Canada, which feeds on <u>Lythrum</u>.

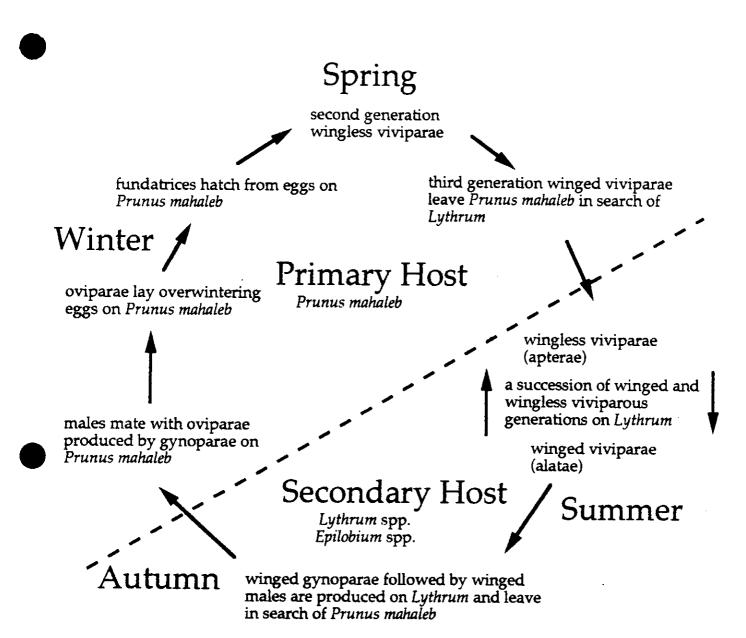
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<u>Myzus lythri</u> came to my attention in the fall of 1992 when Dr. Robert Waltz, State Entomologist for Indiana, asked if I would identify aphids collected on <u>Lythrum salicaria</u> at the Calumet Prairie Nature Preserve in northwestern Indiana. The biologist who had sent them to him had included the following remarks, " Noted as very abundant in sunny areas on <u>Lythrum</u>. Causing stunting, delayed blooming, and death to significant parts of the <u>Lythrum</u> present." I identified the aphid as <u>Myzus lythri</u> (Schrank). A search of the literature revealed that it has been in the United States since the early 1930's (Gillette and Palmer 1934). A statement by Shaposhnikov (1964) notes that this aphid is "sometimes injurious" to <u>Lythrum</u> spp. and is the only reference I could find to suggest that <u>M. lythri</u> is capable of damaging its host. Smith and Parron (1978) list it as being recorded in 13 United States states and 4 Canadian provinces.

<u>Myzus lythri</u> is a host-alternating aphid, migrating from <u>Prunus</u> spp., which function as primary hosts in fall, winter and spring, to <u>Lythrum</u> spp. and

<u>Epilobium</u> spp., which function as secondary hosts in late spring, summer and early fall (Figure 1).

The statement by Shaposhnikov and the occurrence in Indiana suggested that \underline{M} . <u>lythri</u> should be investigated for its potential effect on \underline{L} . <u>salicaria</u>. This aphid was not considered as a potential control agent by either Batra et al. (1986) or Hight and Drea (1991) although it was collected in the surveys for phytophagous insects on purple loosestrife conducted in the northeastern United States (personal communication Manya B. Stoetzel, SEL, USDA, Beltsville). The infrequent occurrence of injury by this aphid was puzzling, but the explanation clearly lies in the life cycle. Three aspects limit the potential of the aphid for biological control under natural conditions. The first is the spring and fall migrations (Figure 1). Any time an aphid flies, it is at the mercy of weather conditions, and spring and autumn weather can be rather rigorous. Yet hostalternating aphids have little choice in the timing of their spring and autumn migrations. While there are no data on mortality during these migrations, it is likely that under inclement conditions it is high. The second factor is the time of migration from Prunus in the spring. In host-alternating aphids of the Aphidinae, most of the third generation on the primary host are winged migrants. In Illinois the spring migration of M. lythri occurs during the first half of June. By this time purple loosestrife has been growing for almost two months and will begin to flower in a few weeks. Although large populations of M. lythri can still develop on L. salicaria, they may be too late to influence flowering and seed set. The third factor is the absence of appropriate primary hosts in the vicinity of most purple loosestrife stands. Without abundant winter hosts in reasonably close proximity to the summer host the survival of large M. lythri populations is not possible. Given these three hurdles, it is not surprising that M.



<u>lythri</u> rarely develops large populations on purple loosestrife early enough in the season to stress the host.

In spite of the well-known ability of large populations to have detrimental effects on their hosts, aphids have generally not been considered as potential candidates for the biological control of weeds. The extensive host range of what might be called "weed aphids" has obscured the fact that the majority of aphid species are quite host specific (Eastop 1986). Aphids have many characteristics which make them good potential candidates for use in biological control. They are highly fecund, gregarious, easy to rear, vagile, feed throughout the growing season and have large numbers of generations annually. <u>Aphis chloris Koch has been introduced into Canada, South Africa and Australia for use in the control of St. John's wort, Hypericum perforatum</u> (Carver 1989). Its release in Australia was to complement introduced chrysomelids (<u>Chrysolina spp.</u>) by feeding on new plant growth after the summer rains when the beetles were not abundant. Oraze and Grigarick (1992) document the ability of the waterlily aphid (<u>Rhopalosiphum nymphaeae</u> L.) to significantly reduce biomass and seedpods in duck salad (<u>Heteranthera limosa</u> (Sw.) Willd.).

Methods

Field Searches for Myzus lythri

In late May 1993, areas in Indiana, Michigan and northern Illinois where purple loosestrife is abundant were visited in an attempt to locate <u>M</u>. <u>lythri</u> on <u>Prunus</u> spp. Additional trips were made throughout the summer and autumn in

an attempt to find the aphid on purple loosestrife. These trips also included parts of Ohio and Wisconsin.

Colonies collected from <u>Prunus mahaleb</u> in May were kept on cuttings at 15 deg. C for the development of spring migrants. As the alatae matured each was placed in a clip cage on a leaf of a large purple loosestrife maintained in the greenhouse. Daily counts of reproduction by these alatae were made and nymphs were removed.

Paired Second Year Plants

Since seeds of L. salicaria were not available, experiments involved the use of second year plants. A large number of second year purple loosestrife were pulled from a flooded gravel bed. Plants were easily removed with most of their root system intact (Figure 2). They were sorted into pairs on the basis of root size and vegetative development and photographed before planting. Plants were potted into standard 15 cm plastic pots in greenhouse potting mix placed on a mist bench for three weeks. Once they were growing satisfactorily, five pairs were chosen based on similarity of growth and structure. Each pair was randomly assigned to control (A) or experimental (B) treatments. Each B plant was inoculated with five 3rd or 4th instar nymphs of M. lythri, and each plant was covered with a cylindrical cage 1.2 m tall. Each pot was placed in a clear plastic saucer which was kept full of water at all times. Plants were maintained at greenhouse conditions for seven weeks when the experiment was terminated. Plants were removed from the pots, root masses soaked and washed carefully to remove all traces of soil. Roots of each pair were photographed, the plants oven dried for one week at 40 deg. C., and weighed.

Seeds became available in the field during late summer 1993 and were started on a mist bed in the greenhouse. After a month they were transplanted into 15 cm pots of silica sand and held in the mist bed until growing well, then transferred to a regular bench. After three months, three sets of ten plants were chosen, each containing an equivalent mixture of seedling sizes. One set served as a control, one set was inoculated with two <u>M. lythri</u> per plant and one set was inoculated with five <u>M. lythri</u> per plant. Plants were individually caged and maintained as in the previous experiment.

Results

Field surveys in Indiana, Michigan and Illinois for <u>M. lythri</u> in May were disappointing. Even in the area where aphids had been in abundance on <u>L. salicaria</u> in the autumn of 1992, I was unable to locate any on <u>Prunus</u> (<u>Prunus</u> <u>serotina</u> and <u>P. virginiana</u>). Aphids were located only at The Morton Arboretum in Lyle, Illinois and only on *Prunus mahaleb*. Of the six trees of this species there, four had colonies of <u>M. lythri</u>. All the other cherry species growing in the arboretum were examined, but <u>M. lythri</u> was not found on any of them. Samples taken on 18 May 1993 consisted of fundatrices, second generation apterae, and nymphs. Collections made on 1 June 1993 from the same trees included fundatrices, second generation apterae and nymphs, and third generation alatoid nymphs and alatae. Alatae from these collections were transferred to purple loosestrife in the greenhouse for colony development.

Collecting trips made in August and September were successful; <u>M. lythri</u> was found almost everywhere that <u>L</u>. <u>salicaria</u> occurred in abundance. All

aphids observed were collected alive during these trips and maintained on cuttings to see if mummies developed. No parasitism was observed in any of the samples. Field observations indicated that <u>Orious insidiosus</u> was the most common predator. A few lacewing larvae and mirid nymphs were also observed. The low predator level probably reflects the small size of the aphid colonies.

Spring migrants from <u>P</u>. <u>mahaleb</u> clip-caged to leaves of <u>L</u>. <u>salicaria</u> settled on the leaf surface after 24-48 hrs and began to feed. There appeared to be no difference in their acceptance of the top or bottom of the leaf surface. The longevity of these alatae varied from 1 to 20 d (mean = 7.62 d, SE = 0.8, n = 45) and reproduction ranged from 0 to 44 nymphs (mean = 11.69, SE = 1.68, n = 45).

Data from the paired plant experiment are shown in Table 1. After five weeks there were visible differences between the control and treatment plants. None of the treatment plants had flowered whereas three of the five control plants had flowered. Two aphid treatment plants showed premature leaf drop, leaving only terminal leaves at the end of seven weeks. The symptoms of stress visible on the shoots were also seen in the roots. The difference in biomass between the control and treatment plants was significant for shoots, roots and total plants.

Data from the seedling test is shown in Table 2. The dry weights are highly variable, especially in the five-aphid treatment. In addition, there was a great difference in the development of aphid populations between the caged plants. Analysis of the data using ANOVA shows a significant treatment effect (p< 0.001) for root size but no significant difference of treatment effect on shoot

(p = 0.187) or total plant weight (p = 0.07). A pairwise comparison of means using the Tukey HSD test shows a significant difference in root weight of control and the two-aphid treatment (p=0.003) and control and five-aphid treatment (p = 0.001). Pairwise comparison of means for shoot and total plant weight showed no significant differences; the closest was a value of p = 0.084 for the comparison of shoot between control and five-aphid treatment.

Plants inoculated with aphids were categorized (post hoc), based on the appearance of the root and shoot, as dying, dead or unaffected. Dead plants were characterized by dead leaves and stem and below ground by dark brown roots. Dying plants showed signs of stress such as smaller, discolored leaves and a mix of healthy (light cream color) and dead roots. Unaffected plants showed no visible changes in shoot or root. Data on these twenty plants were analyzed by ANOVA followed by a Tukey HSD paired comparison of means. A significant affect of category for root, shoot and total plant weight (p < 0.001) was noted. Comparison of paired means is shown on Table 3. Of interest in Table 3 is that there is no significant difference in root weight between dead and dying plants, however, the shoot of the dying plants is significantly larger than the dead plants but not significantly different from those plants considered unaffected.

While this post hoc categorization and analysis may not be statistically valid, the numbers are interesting as they reflect observations made during both the paired plant and seedling tests. Plants seem to show little indication of damage until the point when the plant is essentially covered with aphids. At this time growth virtually stops, leaf drop may occur, and plant color changes from a healthy green to yellow. It appears that stressed plants develop above ground

shoots at the expense of roots. A similar response is seen in the inoculated plants of the paired plant experiment.

Discussion

The clear selection of <u>Prunus mahaleb</u> among all the cherry species at The Morton Arboretum is surprising, given that <u>Prunus virginiana</u>, <u>P. pennsylvanica</u> and <u>P. serotina</u> are all listed as hosts in the literature (Nielsson 1971; Pepper 1965). Of these three only <u>P. pennsylvanica</u> (pin cherry) is in the same subgenus as <u>P. mahaleb</u> (Rehder 1967). <u>Prunus mahaleb</u> is rarely planted as an ornamental but is extensively used in the horticulture industry as root stock for cultivated cherries. As the spring search for <u>M. lythri</u> on native cherries was futile, perhaps searching urban landscapes for cultivated cherries and examining their sucker shoots may have been productive. These sucker shoots may be the over-wintering site for <u>M. lythri</u>. Dr. Susan Halbert (pers. comm.) has been unable to find <u>M. lythri</u> on <u>Prunus virginiana</u> in areas of Idaho where suction traps show large autumn populations of the aphid.

The difficulty in locating <u>M</u>. <u>lythri</u> on <u>Prunus</u> spp. in the spring contrasts markedly to the ease with which it can be found each fall. Field observations suggest a considerable dispersal by alatae occurs throughout the summer. In August, it was common to find small colonies that were undoubtedly initiated by summer migrants from purple loosestrife. In late September, heavy infestations of <u>M</u>. <u>lythri</u> were frequently observed. A successful return migration from these widely disbursed aphid populations to an apparently limited number of primary hosts is unlikely. It appears that the lack of abundant primary hosts in proximity

to purple loosestrife is the annual bottleneck that limits the size of \underline{M} . <u>lythri</u> populations.

The absence of parasitized aphids in field samples is encouraging. It may be that <u>M</u>. <u>lythri</u> has been introduced, much like its host, without its natural enemies. Possibly some native generalist will attack it, as other <u>Myzus</u> spp. have a wide variety of parasites.

In both experiments considerable variability in the rate of development of the aphid population on individual plants was found. This was most likely a function of the success of aphid transfer and possibly the form of the nymphs. Nymphs used in the paired plant experiment were taken from a plant with a low population and consisted primarily of apterous nymphs. The nymphs for the seedling experiment came from a crowded plant and the majority were alatoid. Alatae produce fewer nymphs than do apterae and thus population development may have been delayed. Also, I have found that late instar alatoid nymphs disturb easily, and if disturbed during a moult, often do not satisfactorily complete the moult. The number of aphids per plant in these experiments, however, was less than would be expected to be deposited on a plant by a single alate migrant.

The results of the experiments are not surprising given the reputation of aphids as pests; perhaps this plant response should have been expected. The strong effect on root development is of particular interest especially in a perennial. The possibility also exists that a delay in blooming may limit the number of flowers produced, thereby reducing seed set. It remains to be seen if population densities of aphids observed in these experiments will occur in the

field. In cages, the alatae cannot leave and must settle back on the plant. In the field will there be enough apterae remaining on the plant to maintain a large population? Large populations observed on purple loosestrife in the field in September suggests that the departure of alatae will not limit population growth. Large numbers of aphids and honeydew will also most certainly attract predators that can reduce population size.

With information gleaned from these preliminary studies, it may be possible to manipulate <u>M</u>. <u>lvthri</u> so that it can contribute to the biological control of purple loosestrife. The aphid can be manipulated in two ways. First, mass rearing M. lythri for release in early spring parallels a method used in the biological control of insects (Ridgway and Vinson 1977; Prokrym et al. 1992). Colonies of aphids can be maintained indefinitely under long photoperiod and warm temperature conditions (Blackman 1988). Myzus lythri has shown rapid colony growth under 16 h photoperiod at greenhouse temperature. To estimate the numbers of alatae that could be mass-reared on one large purple loosestrife, two samples were taken from a heavily infested plant in the greenhouse. A 10 cm length of growing tip had 1,300+ aphids; a 10 cm section of stem and leaves lower on the same stalk had 2,600+ aphids. At least 90% of the third and fourth instar nymphs in these two samples were alatoid. Based on these counts, over 20,000 alatae could mature on one large purple loosestrife in a two week period. The use of <u>M. lythri</u> in this manner would be much like a specific time-release herbicide; apply it each spring and it will continue to work through the growing season.

A second method would be to plant <u>Prunus mahaleb</u> trees in close proximity to extensive stands of purple loosestrife in hopes that the natural cycle

will function and maintain a healthy population of <u>M</u>. <u>lythri</u>. Planting an alien cherry would not be appropriate in high quality native wetlands threatened by purple loosestrife, but may be possible to plant trees in adjacent areas. In areas where purple loosestrife occurs in highly disturbed settings, planting large numbers of <u>P</u>. <u>mahaleb</u> would be appropriate.

The potential interaction between the aphids and the leaf feeding beetles (<u>Galerucella calmariensis</u> L. and <u>G. pusilla</u> Duftschmid) presently being released against purple loosestrife (Malecki et al. 1993) must be considered. While there is clear overlap in feeding sites on the leaves, <u>M. lythri</u> also does very well on the stem and it appears unlikely that it would cause significant interference with the beetles.

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Smith, C. F. and C. S. Parron. 1978. An annotated list of Aphididae (Homoptera) of North America. North Carolina Agricultural Experiment Station, Technical Bulletin #255. 428p. Table 1. Comparison of dry weight in grams of root, shoot and total plant of paired Lythrum salicaria seven weeks after B plants were inoculated with 5 aphids each. Paired T-test for roots T= 3.93, DF = 4, P = 0.017; for shoots T = 3.98, DF = 4, p = 0.016; for entire plant T = 4.76, DF = 4, p = 0.009.

<u> Pair #</u>		Plant A	Plant B	<u>B/A</u>
3	Root	9.3	5.5	0.59
	Shoot	9.4	8.8	0.94
	Plant	18.7	14.3	0.7 6
6	Root	12.3	3.2	0.25
	Shoot	8. 9	4.5	0.51
	Plant	21.2	7.7	0.36
8	Root	18.4	3.1	0.17
	Shoot	7.2	4.0	0.56
	Plant	25.6	7.1	0.28
13	Root	11.1	2.0	0.18
	Shoot	6.2	4.3	0.69
	Plant	17.3	6.3	0 .36
19	Root	6.0	1.8	0.30
	Shoot	9.1	4.6	0.51
	Plant	15.1	6.4	0.42

Table 2. Dry weight in grams of roots, shoots and total plant for seedlings after ten week experiment. ¹ Plants classified as dying; ² Plants classified as dead; ³ Plants classified as unaffected.

_	¢	control		2	2-aphid	s	5-a	aphids	;
plant	<u>root</u>	shoot	<u>: plant</u>	root	shoot	<u>plant</u>	<u>root</u>	shoot	plant
1	1.2	6.95	8.15	0.7	5.5	6.21	0.8	5.8	6.61
2	1.1	6.0	7.1	0.8	4.7	5.52	0.9	6.2	7.13
3	2.1	6.8	8.9	1.1	4.7	5.83	0.6	3.8	<u>4.4</u> 2
4	1.8	8.9	10.7	1.5	5.7	7.2 3	0.6	2.0	2.62
5	1.3	5.7	7.0	0.4	2.3	2.72	0.35	2.0	2.35 2
6	1.35	6.7	8.05	0.6	4.5	5.11	0.85	1.6	2.45 2
7	2.15	5.3	7.45	1.05	7.6	8.65 1	0.75	4.9	5.65 2
8	0.95	5.4	6.35	0.3	0.7	1.0 2	0.3	1.4	1.7 2
9	0.9	3.25	4.15	0.75	5.8	6.553	0.5	6.5	7.01
10	<u>1.25</u>	7.0	<u>8.25</u>	<u>0.75</u>	5.5	<u>6.25</u> 3	<u>1.3</u>	<u>10.7</u>	<u>12.0</u> 3
mean	1.41	6.20	7. 6 1	0.80	4.70	5.50	0.70	4.49	5.19
variance	0.20	2.17	2.93	0.12	3.74	4.81	0.086	8.68	10.07

Table 3. Matrix of pairwise comparisons of means using Tukey HSD test for three categories of experimental seedlings inoculated with aphids. Plants dying are category 1, dead plants are category 2, unaffected plants are category 3. Note that root weight is not significantly different between dead and dying and shoot weight is not significantly different between dying and unaffected.

Roots			Shoots			
	1	2	3	1	2	3
1	1.000	-		1.000		
2	0.410	1.000		0.005	1.000	
3	0.111	0.004	1.000	0.894	0.001	1.000

Figure Legends

Figure 1. Outline of the host-alternating life cycle of <u>Myzus lythri</u>. Movement between primary and secondary host in the spring is by winged spring migrants and return from secondary to primary is by gynoparae and males. The gynoparae precede the males to <u>Prunus mahaleb</u> where they produce egg-laying females.

Figure 2. The top row shows five pair of matched second year purple loosestrife. These were potted, allowed to establish and assigned to control (A) or experimental (B) treatments. Experimental plants were each inoculated with 5 aphids. After seven weeks the experiment was terminated, plants were removed from pots and soil washed from the roots. The bottom row shows the roots of control and experimental plants for all five pairs. In pair thirteen A and B plants were photographed in reverse order.

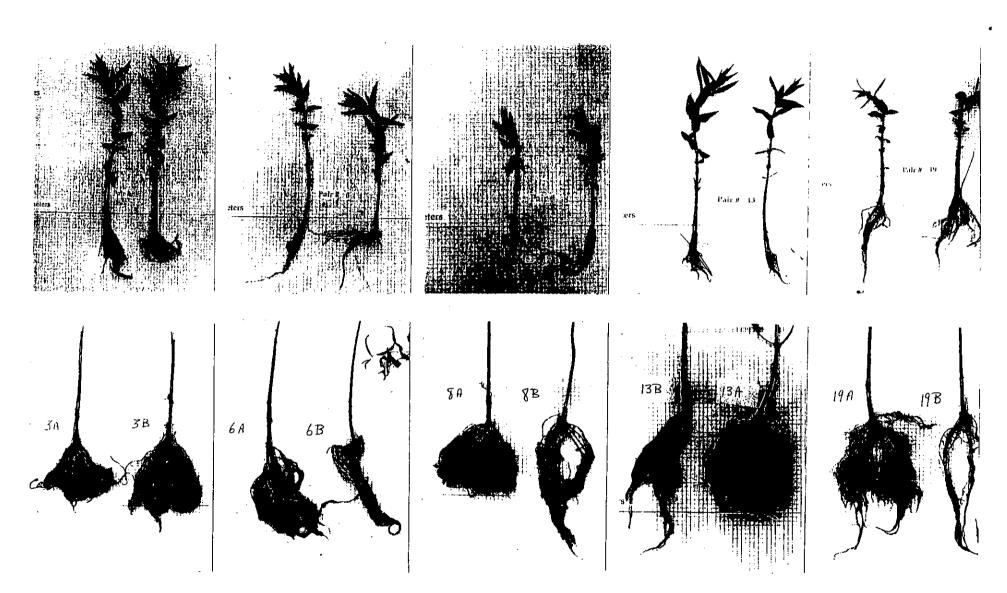


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