somes, corresponded closely with the diploid. The average size of chromosomes in the United States populations (1.67 μm to 5.54 μm; Table 2) was somewhat smaller than that of the diploids from Irish lakes (2.46 μm to 7.79 μ m). Chromosomes of the triploid from Poland were reportedly more condensed than the diploids which may explain the apparent smaller size chromosomes from the United States populations. The size of chromosomes in the Irish lake populations compare well with those of the United States populations in that the group sizes of both idiograms compare proportionately, and the sizes of those from the Irish lakes fall within the ranges observed in the United States populations. Although our idiogram differs from the Polish triploid (6 acrocentric, 15 submetacentric and 3 metacentric chromosomes) we feel that inaccuracies in visualizing and measuring chromosomes can explain these slight differences, and that the idiograms for hydrilla from Poland and the United States can be considered the same.

ACKNOWLEDGMENTS

This research was funded by a grant provided by the Water Resources Research Institute of the University of North Carolina and the Florida Agricultural Experiment Stations.

Appreciation is expressed to Cynthia Smith, Tom Stalker and Don Perry of the North Carolina State University, Department of Crop Science for assistance with karyotyping, Steve Linda, University of Florida, IFAS, Department of Statistics for assistance with statistical analysis and Cindy Dean for typing the manuscript.

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J. Aquat. Plant Manage. 27: 115-118

Laboratory Host Range Studies With a Leaf-mining Duckweed Shore Fly

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ABSTRACT

A leaf-mining shore fly, *Lemnaphila scotlandae* Cresson (Diptera: Ephydridae), collected from duckweeds (*Lemna* spp.) in Florida was exposed in paired-choice oviposition tests to 19 aquatic macrophytes and 1 alga. Eggs were laid only on 6 species in the duckweed family, Lemnaceae. Larvae developed to adults only on 3 of those species, all duckweeds. In no-choice oviposition and fecundity tests, more eggs were laid on common duckweed (*Lemna minor* L.) than on inflated duckweed (*L. gibba* L.) and small duckweed (*L. valdiviana* Phil.). This shore fly can be considered a potential candidate for biocontrol of duckweeds in countries where it is not present.

Key words: Aquatic weeds, biological control, Diptera, Ephydridae, *Lemna*, oviposition tests.

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INTRODUCTION

Duckweeds[3], *Lemna* spp. (Lemnaceae), are small, free-floating macrophytes found worldwide usually on the surface of still or slowly flowing freshwater (Holm *et al.* 1979). Often chemical control methods are not desired and because of their small size, duckweeds are generally difficult to control with mechanical methods. One species, duckmeat (*Lemna perpusilla* Torr.), is considered a noxious weed in Malaysia (Mansor 1987, Mansor and Ahmad 1986) and is thus a potential target for control, especially biological control.

Biological control using insects has been successful against several species of floating aquatic weeds (Julien 1987). This technique is especially apropos in developing nations like Malaysia where costs and environmental concerns preclude traditional chemical control methods. Recently, a highly successful control program was conducted in Papua New Guinea where a floating fern, karibaweed (*Salvinia molesta* Mitchell), was rapidly controlled by a weevil, *Cyrtobagous salviniae* Calder and Sands (Thomas and Room 1986). Control of this fern allowed many residents to return to villages abandoned when the waterways became clogged.

There are no reports of insects being used for biological control of duckweeds (Julien 1987). However, common duckweed in the United States is attacked by several native insects, including a leaf-mining shore fly, *Lemnaphila scotlandae* Cresson (Ephydridae) (Scotland 1940). The larvae of this fly kill duckweed leaves by eating their entire contents leaving behind transparent, hollow shells. Three species of native parasites, small wasps, attack the immatures in the United States (Scotland 1940). These wasps greatly reduce the fly populations thus moderating the effects of these flies on duckweeds. Unlike most flies, adults of this species feed on the plant. They extensively damage duckweed by making parallel gouges in leaves. This damage and the rasping mouthparts have been illustrated by Scotland (1940).

The only reported field host plants of *L. scotlandae* are common duckweed (Scotland 1940), inflated duckweed, and small duckweed (Buckingham 1989). This field specificity suggested that the fly might be safe for use as a biological control agent. We initiated this study of the laboratory host range to confirm that the fly is indeed specific to duckweeds and to stimulate interest in it as a potential agent for control of duckweed in Malaysia.

MATERIALS AND METHODS

All experiments were conducted at the Biological Control Laboratory, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, from February through June 1987. Adult flies were collected when needed from small duckweed in water tanks outside the laboratory. Additional flies were obtained from field-collected common duckweed and inflated duckweed.

Paired-choice Oviposition Tests

Nineteen aquatic macrophytes and one alga were used in the oviposition tests (Table 1). They were chosen either because they were in the host plant family, Lemnaceae (seven species); were in Araceae and Typhaceae, families believed closely related to the Lemnaceae (four species);

TABLE 1. SUMMARY OF PAIRED-CHOICE OVIPOSITION TESTS WITH *LEMNAPHILA SCOTLANDAE*.

Test Plant		Test	Amount of
Family species	Common Name	Symbol[a]	Oviposition[b]
Amaranthaceae			
Alternanthera philoxeroides (Mart.) Griseb.	alligatorweed	A	0
Apiaceae			
Hydrocotyle umbellata L.	water pennywort	B	0
Araceae			
Colocasia esculenta (L.) Schott	taro	C	0
Orontium aquaticum L.	goldenclub	D	0
Pistia stratiotes L.	waterlettuce	E	0
Azollaceae			
Azolla caroliniana Willd.	Carolina mosquitofern	F	0
Cladophoraceae			
Pithophora sp.	alga	G	0
Haloragaceae			
Myriophyllum aquaticum (Vell.) verdc.	parrotfeather	A	0
Hydrocharitaceae			
Limnobium spongia (Bosc) Steud.	American frogbit	H	0
Lemnaceae			
Lemna gibba L.	inflated duckweed	D,I,J,K	+ + +
Lemna minor L.	common duckweed	G,I,L,M	+ +/+ + +
Lemna valdiviana Phil.	small duckweed	C,E,M,N	+ +/+ + +
Spirodela polyrhiza (L.) Schleid.	giant duckweed	N,O	+
Spirodela punctata (Meyer) Thomps.	—	L,O	+
Wolffia columbiana Karst.	common watermeal	K,P	+
Wolfiella floridana (J. D. Smith ex Hegelm.) C. H. Thomps.	flat bogmat	P	0
Poaceae			
Oryza sativa L.	rice	F	0
Pontederiaceae			
Eichhornia crassipes (Mart.) Solms	waterhyacinth	B	0
Salviniaceae			
Salvinia minima Baker	water fern	H	0
Typhaceae			
Typha latifolia L.	common cattail	J	0

[a]Plant species with the same letter were tested together.
[b](0) = no oviposition, (+) = ≤0.5 eggs per female per day, (+ +) = 0.6 to 1.0 eggs per female per day, (+ + +) = 1.1 to 6.9 eggs per female per day.

[3]Common and scientific names of the major plants discussed in this paper are listed in Table 1.

were economically important (rice); or were readily available for testing (eight species).

A 29.6-ml plastic cup filled two-thirds with tap water was used in these tests. Test plants, or disks and sections of different sizes cut from the larger plants, were floated on the surface to cover approximately the entire surface area, about 9.6 cm^2. Two plant species were paired in each test. Three cups were exposed together in a 950-ml vented plastic cylinder (11 cm diameter, 10 cm height) containing about 3 cm of water which was approximately as deep as that in the cups. One cup contained species A, one contained species B, and the third contained an equal mixture of A and B. Three 1 to 2-day-old female flies were released into the cylinder which was immediately capped. Each test had 3 replicates, and some plant species were tested several times. Tests were run for either 2 or 3 days in a temperature cabinet at constant 27 ± 2C and 16-hr photophase. At termination eggs were counted from each cup. For calculation of the number of eggs per female per day the assumption was made that all deaths occurred shortly before termination. Means for the duckweed pairs were compared with a paired t-test ($p \leq 0.05$) using data transformed with $\sqrt{x + 0.5}$ because of the large number of zeroes and small values. All eggs were subsequently used in the larval development tests.

No-choice Oviposition and Adult Feeding Tests

Test 1. Individual 1- to 2-day-old females were exposed to either common duckweed, inflated duckweed, or small duckweed in the cylinder with three cups as described previously; however, all cups in a cylinder had the same plant species. This test arena was chosen because preliminary studies showed that mortality was high when flies were confined directly in the small cups and because there was too much plant material for examination if the entire water surface in the cylinder was covered with plants. Eggs and leaves with adult feeding scars were counted in each container after two days. There were three replicates of each plant species. One-way analyses of variance (ANOVA) were calculated from means of eggs per female transformed with $\sqrt{x + 0.5}$ because of small values and from means of percent of leaves with feeding scars transformed with arcsin $\sqrt{x}$.

Test 2. A subsequent test was conducted like Test 1, but the plants in each cup were replaced daily for three days to minimize possible negative effects of accumulative feeding damage on adult feeding and oviposition behavior. The cups were held without examination after day 3 until all adults had died on day 8 when eggs were counted. Time constraints at the end of this study prevented testing third replicates of common duckweed and small duckweed and observation of two replicates of inflated duckweed on day 3.

No-choice Larval Development Tests

Leaves with eggs deposited in the paired-choice oviposition tests were separated by species into cups and held for larval development. Adults were counted as they emerged.

Common duckweed, inflated duckweed, and small duckweed, all of which supported larval development in the preceding test, were compared. Eggs were obtained from colony females held on the respective plant species. Five eggs on leaves of one of these species were placed into a cup and additional leaves of the same species were added to cover the surface. The cups were either individually capped or placed together into larger closed containers of various types to capture emerging adults. Empty puparia in the test plants were counted to confirm the number of adults emerged from each plant species. Cups were held either in a temperature cabinet at 27 ± 2C, 16-hr photophase, or in a greenhouse with a usual temperature range of 24-30C and natural lighting with 16-hr supplemental fluorescent lighting. The number of cups with five eggs each for each test plant were as follows: common duckweed 26, inflated duckweed 10, small duckweed 12. In addition, several cups were initiated with larger numbers of eggs per cup. They were common duckweed, 3 cups (10 eggs each), inflated duckweed, 1 (10), 1 (20); small duckweed, 1 (10), 1 (50). Forty eggs were tested on common duckweed in a 0.9L jar.

RESULTS AND DISCUSSION

Paired-choice Oviposition Tests

Most eggs were laid on the three species of duckweeds (Table 1), but a few were laid on the two species of giant duckweeds and on common watermeal. None were laid on plant species outside the Lemnaceae in any of the tests. There was no preference when common duckweed was paired with small duckweed, 4.8 ± 0.7 vs 3.9 ± 1.3 eggs per female per day, but inflated duckweed was preferred when paired with common duckweed, 5.4 ± 1.1 vs 3.2 ± 0.9 eggs per female per day.

No-choice Oviposition and Adult Feeding Tests

In Test l, common duckweed stimulated the most eggs per female per day and inflated duckweed the least (Table 2). The same order was found for adult feeding. These results, although not statistically significant, suggest that the apparent ovipositional preference for inflated duckweed over common duckweed observed in the paired-choice test may have been a negative response by females to greater accumulative feeding damage on common duckweed rather than a true preference for inflated duckweed. Casual observations during this study suggested that females oviposited most heavily on nondamaged or little-damaged plants.

Oviposition and feeding behavior were also examined in Test 2 in which leaves were replaced daily for three days. Again, common duckweed received the most eggs per female per day, 13.2 (13.0 & 13.3), but inflated duckweed, 6.2 ± 0.6 (range 5.5-6.7), and small duckweed, 5.5 (5.3 & 5.7), reversed their order. Total fecundity over the eight-day period was highest on common duckweed, intermediate on inflated duckweed, and lowest on small duckweed (Table 2). There was a greater difference in total fecundity than in 3-day fecundity between small duckweed and the other species. This may reflect a nutritional inadequacy of small duckweed for egg production. The percentage of leaves of each species damaged on the

TABLE 2. SUMMARY OF NO-CHOICE TESTS WITH FEMALES AND LARVAE OF *LEMNAPHILA SCOTLANDAE*.

Test Plant	Oviposition[a] (Eggs/ Female/Day)	% Leaves With[b] Feeding scars	Fecundity (Total Eggs/ Female)	% Larvae Developed to Adults
Common Duckweed	10.4 ± 3.0 (7.0-12.5)	29.5 ± 12.0 (21.7-43.3)	80.0 (77.0&83.0)	35.4
Inflated Duckweed	2.9 ± 1.3 (1.5-4.0)	17.5 ± 6.7 (10.0-23.0)	39.0 ± 9.0 (30.0-48.0)	30.0
Small Duckweed	5.5 ± 3.8 (2.0-9.5)	28.5 ± 17.0 (17.5-48.1)	16.5 (16.0&17.0)	10.0

[a]$\bar{x} \pm$ SD (range), ANOVA, $p = 0.051$
[b]ANOVA, $p = 0.45$

first, second, and third days were: common duckweed, 33.0, 31.5, 12.5%; small duckweed, 25.5, 41.0, 23.5%; and inflated duckweed, 12.3, 8.0, 19.0%. These daily percentages were similar to those found after 2 days in Test 1. This suggested that females concentrated their feeding on already damaged leaves after first exposure instead of feeding on new leaves.

Mortality was recorded for some adults although no tests of adult longevity were conducted. Adults lived as long as 6 days and frequently for 4.5 days. This was considerably longer than the 2.5 day (60 hr) maximum reported by Scotland (1939).

No-choice Larval Development Tests

From the eggs deposited on test plants in the paired no-choice oviposition tests, larvae developed to adults only on common duckweed, small duckweed, and inflated duckweed. None developed from 20 eggs on *S. punctata*, from 4 eggs on giant duckweed, or from 13 eggs on common watermeal. Because female oviposition was so specific, no egg transfer tests were conducted. Neonates burrowed directly through the bottoms of the eggs into the leaves; thus, plants without eggs would not appear to be at risk from neonates.

In the larval development tests (5 eggs/cup), common duckweed and inflated duckweed produced similar numbers of adults with small duckweed being a marginal host (Table 2). Interestingly, small duckweed was host for the colony in our outdoor laboratory tanks; however, the colony was never large. Larval success was lower when more than 5 eggs were tested per cup. The success rates, similar for all three plant species at these higher densities, were 10-13% with 10 eggs per cup and 2.0-7.5% with 20 or more eggs per container.

Developmental times ($n = 22$) were monitored in one greenhouse test with common duckweed. The first adults emerged on day 14. Sixty-four percent emerged by day 20, and 96% by day 23. The last adult emerged between observations on day 23 and day 28.

Our tests have confirmed that *L. scotlandae* is sufficiently specific to warrant additional testing within countries interested in biological control of duckweeds. Females laid eggs primarily on duckweeds and no adults developed from the few eggs laid on closely related plants. Flies developed in all three species of duckweeds tested, but common duckweed appeared to be the most suitable host. Before initiating an intensive study, researchers in Malaysia or other countries should determine in a secure laboratory whether this fly will accept the target duckweed and whether it will attack other native species in the Lemnaceae.

Most reports of this fly are from northern locations in the United States, which suggests that it might be unsuitable for tropical climates. However, it has recently been found in southern Florida (Buckingham 1989) and thus may have a wider climatic tolerance than previously thought. Additional insects attack duckweeds in the tropics, for example, *Lemnaphila neotropica* Lizarralde de Grosso, one of three species in the genus from South America, which has been reported from *Lemna minima* Philippi in Argentina, Panama, and Jamaica (Lizarralde de Grosso 1978) and a related shore fly, *Hydrellia williamsi* Cresson, from Hawaii (Williams 1938). An additional species destructive to duckweeds in the United States is a leaf-mining weevil, *Tanysphyrus lemnae* (F) (Scotland 1940). Often a complex of several species of insects is introduced during a biocontrol program because it is difficult to predict which agents will be successful (Buckingham 1984).

ACKNOWLEDGMENTS

Initial drafts of the manuscript were reviewed by Kim Haag and Paul Boldt. We would like to thank them as well as the following individuals who helped by providing infested plants, field support, or in numerous other ways: Chris Bennett, Margaret Glenn, Kim Haag, Bill Haller, Joe Joyce, Terry Lott, and Emmanuel Okrah. Funds were provided through a Fulbright Fellowship to the senior author. The Center for Aquatic Plants, IFAS, University of Florida, hosted the senior author during his stay in Florida. Florida Agricultural Experiment Station Journal Series No. 9785. Received for publication February 27, 1989 and in revised form May 5, 1989.

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