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NEOMUSOTIMA FUSCOLINEALIS (LEPIDOPTERA: PRYALIDAE) IS AN UNSUITABLE BIOLOGICAL CONTROL AGENT OF *LYGODIUM JAPONICUM*

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ABSTRACT

In its native Japan *Neomusotima fuscolinealis* Yoshiyasu feeds on and damages the leaves of *Lygodium japonicum* (Thunberg ex Murray) Swartz, an invasive weed in Florida and the southeastern U.S. Larvae and pupae of the moth were imported into the quarantine facility at the Florida Biological Control Laboratory, Gainesville, Florida, to establish a colony for preliminary host range studies and to define its lifecycle and reproduction parameters. Larvae of the moth did not feed significantly nor develop on 5 tested rare, native Florida ferns. The rare North American native climbing fern, *Lygodium palmatum* (Bernhardi) Swartz, however, supported complete development of *N. fuscolinealis*, and 6 continuous generations of the moth were reared on the fern. Because the rare *L. palmatum* and the invasive *L. japonicum* co-occur in the US, the release of *N. fuscolinealis* could result in the harm to *L. palmatum*, a risk that makes the moth unsuitable as a potential biological control of *L. japonicum*.

Key Words: *Lygodium palmatum*, Florida, rare native ferns

RESUMEN

Neomusotima fuscolinealis Yoshiyasu es una polilla indígena del Japón donde se alimenta y daña las hojas de *Lygodium japonicum* (Thunberg ex Murray) Swartz, una maleza invasora en la Florida y en el sureste de los Estados Unidos. Larvas y pupas de la polilla fueron importadas a la facilidad de cuarentena en el Laboratorio de Control Biológico de Florida, Gainesville, Florida, para establecer una colonia para realizar estudios preliminarios del rango de hospederos, definir el ciclo de vida y sus parámetros de reproducción. Larvas de la polilla no se alimentaron significativamente, ni se desarrollaron sobre los 5 helechos raros y nativos de la Florida probados. Sin embargo, el helecho trepadora raro y nativo para norteamerica, *Lygodium palmatum* (Bernhardi) Swartz, sostuvo el desarrollo completo de *N. fuscolinealis* y 6 generaciones continuas de dicha polilla fueron criadas sobre el helecho. Debido a que el helecho raro, *L. palmatum* y *L. japonicum* (un helecho invasor) coexisten en los Estados Unidos, la liberación de *N. fuscolinealis* puede resultar en un daño a *L. palmatum*, un riesgo que hace la polilla del Japón inapropiada como un candidato potencial para el control biológico de *L. japonicum*.

Lygodium japonicum (Thunberg ex Murray) Swartz), Japanese climbing fern, is rapidly spreading from northern Florida into central and southern regions of the state. First introduced into the United States as an ornamental around 1900, populations of Japanese climbing fern are now present in nine states from the Carolinas through Georgia, Florida, Alabama, Mississippi, and Louisiana to Texas and Arkansas (Nauman 1993; Langeland & Burks 1998). In Florida, *L. japonicum* has been reported in 53 of 67 counties (Van Loren 2006).

Lygodium japonicum is a perennial fern with wiry brown rachis and yellowish green leaflets or pinnules capable of forming thick mats that can shade and eliminate underlying vegetation. (Nauman 1993). *Lygodium japonicum* is similar in appearance to *L. microphyllum* (Canvanilles) R. Brown, the other more invasive climbing fern that is a problem in more moist habitats of southern and central Florida. The margins of the sterile leaflets of *L. japonicum* are toothed compared with the entire margins of *L. microphyllum*.

A decision was made to develop a biological control project for the Old World climbing fern (*L.microphyllum*) in 1997 (Pemberton et al. 2002), but not for *L. japonicum* because the weed is partially sympatric with the rare native congener *L. palmatum* in its range in the eastern US. Our concern was, and is, that biological control agents developed for *L. japonicum* might harm the rare *L. palmatum*. An opportunity to test that possibility arose when an herbivorous moth of *L. japonicum* was discovered in Japan.

On Oct 3, 1997 R. W. Pemberton discovered larvae of a pyralid moth, subsequently determined to be *Neomusotima fuscolinealis* Yoshiyasu, feeding on the fronds of *L. japonicum* plants in 3 areas of the Tokyo Botanical Garden in Japan. The young larvae fed gregariously, skeletonizing the leaflets, and older larvae completely consumed the blade tissue of the leaflets (pinnae). Pupae were found within the webbing and frass associated with feeding larvae. Two infested *L. japonicum* plants were growing in a shade and glass house occupied by a diverse planting of fern species. Searches of these other ferns failed to yield either *N. fuscolinealis* larvae or the characteristic damage, suggesting that the moth might have a high degree of host specificity. On Oct 7, the larvae were found feeding on *L. japonicum* vines in the Honda section of Tsu City in Mie Prefecture about 300 km west of Tokyo. The characteristic damage by the moth was also found on *L. japonicum* growing in Ugata-Yokoyama Park, s. of Ise in Mie Prefecture. Larvae from Tokyo were fed leaves of *L. japonicum* and reared to the pupal stage. Specimens of reared adults were determined to be *N. fuscolinealis* by lepidopterist Yutaka Arita, of Meijo University, Nagoya, Japan. The immature stages and host plant of this moth were previously unknown (Y. Arita, pers. comm.).

On a subsequent trip to Japan in Jun 2002, *N. fuscolinealis* was sought in the Tokyo Botanical Garden and the Mie locations but was not found. In Sep 2002 larvae and pupae were collected in the Tokyo Botanical Garden and shipped to the U.S. for biological studies and preliminary host range tests focusing on the native North American *L. palmatum*. *Lygodium palmatum* is a temperate plant that ranges north to New York along the Eastern seaboard, but is generally local and rare except on the Cumberland Plateau of Kentucky and Tennessee (Nauman 1973). Thus, the weedy *Lygodium* and the rare *Lygodium* are sympatric in the upper South and the Carolinas. If preliminary feeding tests in quarantine demonstrated that *L. palmatum* could not support the development of *N. fuscolinealis*, then additional host range research would be conducted to more precisely determine its potential host range in North America.

Materials and Methods

All research was conducted at the quarantine facility at the Florida Biological Control Laboratory, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, from Sep 2002 to May 2003. *Lygodium japonicum* plants were collected in Hamilton, Suwannee, and Madison counties, Florida by Dr. Min Rayamajhi, USDA-ARS, IPRL, Ft. Lauderdale, Florida, and *L. palmatum* plants were collected in Rome Co., Tennessee, by the senior author. A shipment of 40 *N. fuscolinealis* larvae and pupae was received from Japan on Sep 30, 2002 collected by Tokyo Metropolitan University colleague Akira Shimizu and students at the Tokyo Botanical Garden site.

First generation adults were transferred to a screen sleeve cage ($30.5 \times 30.5 \times 30.5$ cm) containing a bouquet of cut sprigs of *L. japonicum* and held in a quarantine laboratory at 22°C. Adults were fed 10% honey-Gatorade solution provided on a rolled dental cotton wick in a 7-dram glass vial filled with the solution. A bouquet of *L. palmatum* was added, and then bouquets of both plants were changed every 3-4 d until the adults died. Bouquets with eggs were removed from the cage and separated by plant species into $28 \times 15 \times 5$ cm translucent plastic rearing boxes. The boxes were checked daily for egg hatch and larval development, and fresh plant material was added when necessary until adult emergence.

After establishment, the colony was held in the greenhouse at an average temperature of 23°C, and natural light supplemented by fluorescent lights with a photoperiod of 16:8 (L:D). Adults were exposed to plants of both species together in wooden sleeve cages $47.0 \times 55.0 \times 47.0$ cm or $54.6 \times 72.4 \times 44.5$ cm until the adults died. To conserve *L. palmatum* plants, only 50 eggs on each of 3 plants of each *Lygodium* species were allowed to hatch. Larvae were allowed to feed freely on the plant of their choice until pupation, and plants with pupae were caged separately. New adults emerging from each species were counted and sexed with aid of a stereomicroscope and placed back in the sleeve cages on fresh plants of both species.

As the experiment progressed and the supply of *L. palmatum* plants dwindled, the number of eggs allowed to develop on each plant had to be further reduced to 25 per plant on 3 plants of each *Lygodium* species.

Biological Studies

All studies were conducted in environmental chambers at 26.7°C, 75% RH, and a photoperiod of 16:8 (L:D), unless otherwise indicated. Individual larvae on Japanese climbing fern were confined in plastic boxes with lids, $8.0 \times 8.0 \times 5.0$ cm. The boxes were examined daily during the lifetime of the experiment for larval development, pupation, and adult emergence.

Newly emerged adults were paired in plexiglass cylinders (42 cm high, 14.5 cm ID) with a bouquet of *L. japonicum* sprigs to determine fecundity and adult longevity. The bouquets were changed daily, and eggs were counted and cut from the leaflets. Eggs on the small pieces of leaf material were then placed on moist cotton in 30-ml clear plastic cups capped with a translucent plastic lid with a cotton-plugged hole and checked daily for eclosion.

To determine whether adults could survive cold temperatures, 5 males and 5 females were placed individually in 15-mL plastic vials with no paper or plant material. The vials were then placed in a household refrigerator freezer at -4°C for 24 h.

Multi-choice Larval Host Range Studies

The plastic boxes used in the biological studies were also used in a multi-choice host range experiment with medium sized larvae. Approximately 900 mm^2 (30 × 30 mm) of fronds from 5 native species, 4 of which are listed as endangered by the Florida Department of Agriculture (Nelson 2000), were tested together. A companion control treatment had an equal amount of *L. japonicum*, 4500 mm^2. Each test had 5 replications of each treatment each with 3 larvae randomized to treatments in groups of 3. The replicates were randomized to positions on a laboratory table. After 3 d, the number of living larvae was recorded and feeding on the test plants was estimated with a 1-mm^2 grid. Feeding on host plants was estimated by counting the number of leaves and multiplying by an estimated mean leaf area. The estimated mean area was determined by measuring *L. japonicum* leaflets with a Li-Cor Portable Leaf Area Meter, model Ll-3000. Fifteen samples of leaflets, each approximately 900 mm^2, were measured and a mean leaf area was determined.

One host range experiment was conducted in the small screen sleeve cage in a quarantine greenhouse. Bouquets of *L. microphyllum* were exposed to *N. fuscolinealis* adults for 4 d. Plant material with eggs was then placed in the plastic translucent rearing boxes and held for adult emergence.

Results and Discussion

Results of the rearing experiments are summarized in Table 1. Fourteen females and 2 males emerged from the pupae sent from Japan. These insects were exposed to bouquets of *L. japonicum* and *L. palmatum* and initially all the eggs but one were deposited on Japanese climbing fern. One hundred and forty females and 105 males emerged from *L. japonicum* compared to no adults from the *L. palmatum*. These adults were then divided into 2 cages with bouquets of both plant species. Adults were produced on both species of plants. The experiment continued for 6 generations, with the result that adults produced on *L. japonicum* were 1,202 females and 979 males compared to 249 females and 230 males produced on *L. palmatum*. It was concluded after the 6th generation that *L. palmatum* could support complete development of *N. fuscolinealis* and the insect was not specific enough to be considered as a biological control agent of Japanese climbing fern.

Biological Studies

The moths mate shortly after emergence, usually at night. Females lay the eggs on both sides of the leaflets usually the second night after emergence. The eggs initially are flat, but swell as they mature and the developing larva is visible through the chorion prior to egg hatch. The duration of the egg stage averages 9 d. Larvae are yellowish-cream to green in color with black spots and dark brown heads. The young larvae feed by scraping the surface of the new growth, while older larvae eat the entire leaflet including older and fertile leaves. The duration of the larval stage is 11.2 ± 1.1 d (n = 21). The mature larva spins a very loose web of silk on the leaflet and rachis and forms a brown naked pupa beneath it. The development time for the pupal stage is 5.7 ± 0.6 d (n = 21).

The adults are brown with distinctive white boomerang-shaped marks near the margin of the forewing. The development time from neonate to adult at 26.7°C is 16.9 ± 1.3 d (n = 21). None of the individual insects were followed from egg deposition to adult, so the total developmental time was

Table 1. *Neomusotima fuscolinealis* adults produced on two *Lygodiums*.

	Lygodium japonicum		*Lygodium palmatum*	
Generation	Females	Males	Females	Males
Parents	14	2	0	0
F1	140	105	0	0
F2	171	208	113	118
F3	136	96	79	55
F4	278	179	37	37
F5	259	230	13	16
F6	264	159	7	4
Total	1262	979	249	230
Means	180	140	36	33
SD	95	78	44	43

TABLE 2. MULTI-CHOICE TEST OF *NEOMUSOTIMA FUSCOLINEALIS* LARVAE WITH RARE NATIVE FERNS.[1]

Plant species	Living larvae		Ferns eaten (mm^2)	
	Mean	SE	Mean	SE
Actinostachys pennula	1.2	0.3	0.0	0.0
Anemia adiantifolia	1.2	0.3	0.4	0.2
Ctenitis sloanei	1.2	0.3	0.0	0.0
Tectaria fimbriata	1.2	0.3	4.6	2.7
Tectaria heracleifolia	1.2	0.3	25.4	9.0
Lygodium japonicum	2.2	0.1	224.9	53.3

[1]First 5 species tested together in same cage with 900 mm^2 of cut fronds of each species except *A. pennula*, which had 100 mm^2. An equal amount of *L. japonicum*, 4,500 mm^2, was in each control cage. There were 3 medium-sized larvae in each cage for 3 d and 5 replications each of the treatment and control.

estimated. Total developmental time at 26.7°C should be 24-30 d based upon an egg stage of 9 d and the larval/adult times.

In oviposition studies, females laid an average of 146.6 ± 84.0 (2-233) eggs and 68.8 ± 24.3% of the eggs hatched. Female longevity averaged 6.9 ± 2.6 d (n = 12) compared with the male at 11.1 ± 2.2 d (n = 9).

One female survived 24 h at -4°C without plant material or moisture. After removal from the freezer, the female survived 2 more d in a cage in the greenhouse.

Multi-choice Tests

The results of the multi-choice test with a companion control are presented in Table 2. There was no feeding on *Actinostachys pennula* (Swartz) Hooker and *Ctenitis sloanei* (Poeppig ex Sprengel) C.V. Morton. Feeding on *Anemia adiantifolia* (Linnaeus) Swartz was minimal, averaging 0.4 mm^2. The 2 species of *Tectaria* were both attacked, but *T. heracleifolia* (Willdenow) L. Underwood suffered heavier damage than the related *T. fimbriata* (Willdenow) Proctor & Lourteig. However, the feeding on both species of *Tectaria* was slight when compared with the feeding on the control.

Females oviposited on bouquets of *L. microphyllum* in a sleeve cage in the greenhouse. Sixty six adults were produced in the first generation and 77 adults in the second generation.

Neomusotima fuscolinealis was eliminated as a potential biological control agent of *L. japonicum* because of its potential use of the rare native *L. palmatum*. Our studies demonstrated that the moth could complete its development on the plant for multiple generations.

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