Laboratory Rearing and Life History of *Arzama densa*,[1] a Potential Native Biological Control Agent Against Waterhyacinth

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ABSTRACT

The native moth, *Arzama densa*, a biological control agent against waterhyacinth, was reared in the laboratory on a modified wheat germ-casein diet. Field-collected larvae placed on the artificial diet provided the stock colony for three consecutive laboratory generations. Laboratory-reared pupae were equal to, or significantly larger than, field-collected pupae. Adults reared from artificial diet-fed larvae developed normally and their rate of reproduction compared favorably with that of adults acquired from prepupae or pupae collected in the field. Augmenting natural populations of the moth with laboratory-reared individuals appears feasible since 200 females of the F_3 laboratory-reared generation produced about 51,000 fertile eggs.

INTRODUCTION

Waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) is a perennial herbaceous, floating freshwater weed. It seasonly infests about 400,000 hectares of water bodies including rivers, canals, streams, reservoirs and coastline areas in the southeastern United States. The weed has been ranked eighth among the world's worst weeds and as the most important aquatic weed (12, 13). Waterhyacinth mainly reproduces asexually by stolons and new plants form at their tips (1). Penfound and Earle (15) reported that doubling time in numbers of individuals to be 11 to 15 days, depending upon weather conditions. Such growth potential allows the weed to infest new areas very quickly and form dense, floating mats of plants. Major agricultural, navigational and health-related problems arise from the floating vegetation. Waterhyacinth reduces the oxygen concentration of the water (21), impedes water flow (8), restricts commercial and recreational water traffic (25) and serves as a refuge for insect vectors of human and animal diseases (2). Although it is difficult to arrive at a monetary estimate of damage caused by waterhyacinth and by its control, studies indicate annual figures in the millions of dollars (19, 20).

Chemical, biological, ecological and mechanical methods of control have been attempted in an effort to reduce the spread and the losses caused by the plant (16, 17). Cost, feasibility and environmental effects have played important roles in partial success and/or failure of these control methods.

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This paper describes a study of the biological control of waterhyacinth using a native moth, *Arzama densa* (24) (Figure 1). Previous studies on the life history of *A. densa* indicate that the larvae severely damage the terminal bud and crown portion of waterhyacinth (Figure 2), and pickerelweed (*Pontederia cordata* L.) (3, 4, 22, 23).

Since this insect shows potential as a biological control agent, this study describes an artificial diet and the technology necessary for establishing and maintaining a laboratory colony. The final goal will be to augment laboratory-reared larvae or other developmental stages into natural infestations of waterhyacinth.

MATERIALS AND METHODS

Contents and Preparation of Diet. Waterhyacinths were collected in the field from Venice, LA and reared in the greenhouse at the Delta States Research Center at Stoneville, MS. Greenhouse plants were grown in pools of water containing a 10% modified Hoagland's solution (5, 11). Roots and flowers were removed, leaving the crown, leaves and leaf and flower stems which were rinsed in distilled water and freeze-dried in a Virtis® freeze dryer. The plant material was ground in a Ball mill into a fine powder. The waterhyacinth powder was substituted for alfalfa used in a cabbage looper diet (9, 14) and other changes included the addition of more water, a different vitamin mixture and autoclaving part of the diet (Table 1).

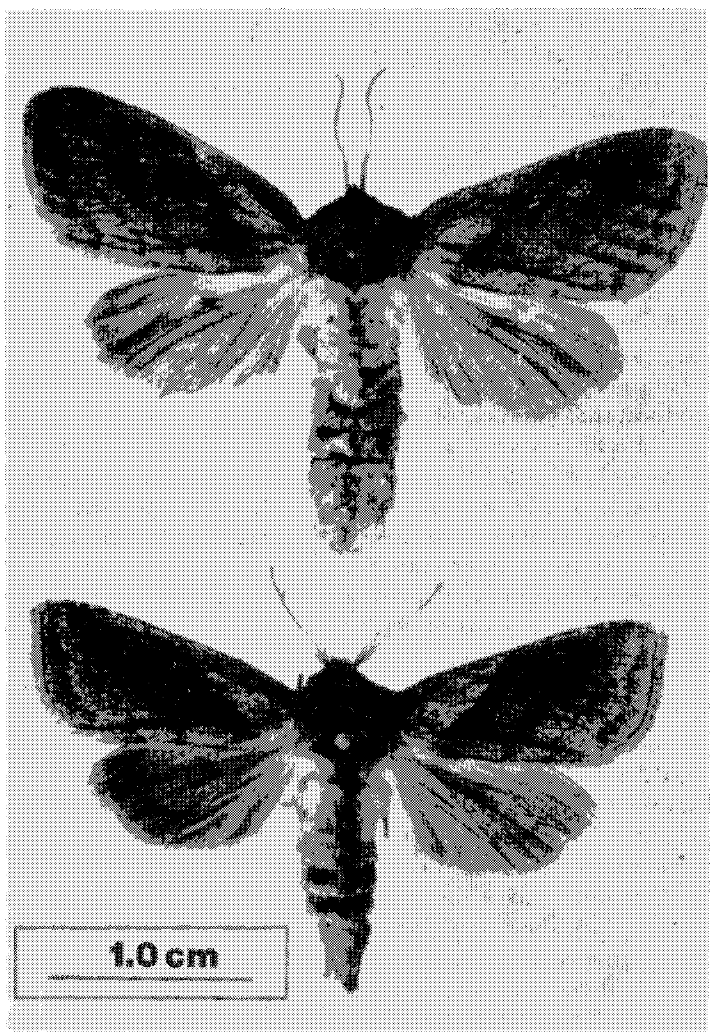

Figure 1. Dorsal view of *Arzama densa,* ♀ top, ♂ bottom.

Figure 2. *Arzama densa* 7th instar larva feeding in the crown of waterhyacinth.

Before diet preparation, the Vanderzant-Adkisson diet and the agar were autoclaved at 17.6 kg/cm^2 for 20 min. The agar was dissolved in 4000 ml boiled water in a blender for 30 sec. The waterhyacinth powder and the V-A diet were added and blended for 1 min. Sorbic acid, choline chloride, formaldehyde, methylparaben and potassium hydroxide were added and also blended for 1 min. The 300 ml suspension (ascorbic acid, aureomycin, vitamin A and vitamin mix) was added and the mixture was blended for 2 min to obtain a homogeneous mixture. About 20 ml of the liquified diet was then poured into 30-ml clear plastic cups, or 35 ml into 100x15 mm plastic petri dishes under a microbiological hood. The diet was stored in a refrigerator at 3±2 C and warmed to room temperature as needed for the developing larvae. The listed quantities of constituents provided enough diet for ca 225 cups or 130 petri dishes.

TABLE 1. AN ARTIFICIAL DIET FOR REARING *Arzama densa* IN THE LABORATORY.

Constituents	Amounts Vanderzant-Adkisson Insect Diet/Modified[a] (400 g)
Wheat germ	96 g
Casein (vitamin free)	112 g
Sucrose	112 g
Salt mix wesson modified[b]	32 g
Cellulose	47 g
Linseed oil	0.8 ml
Cholesterol	0.2 g
Waterhyacinth powder	55 g
Agar	90 g
Sorbic acid	6 g
Choline chloride (10%)	36 ml
Formaldehyde (10%) (37% actual)	15 ml
Methylparaben (38% in 95% ETOH)	18 ml
Potassium hydroxide (4.0 M)	18 ml
The following suspended in 300 ml distilled water:	
Ascorbic acid	15 g
Aureomycin	0.5 g
Vanderzant vitamin mix/modified[c]	9 g
Vitamin A acetate	0.5 g
Distilled water	4000 ml

[a,b,c]Obtained from Bio-Serv, Inc., P.O. Box 100-B, Frenchtown, NJ 08825.

Rearing Procedure. The stock colony of *A. densa* was obtained from waterhyacinth in Venice, LA during July 30-August 1, 1979. *A. densa* populations were highest 1 to 2 m from the shoreline and near overhanging vegetation. Larvae were removed from the leaf petioles or crown portion of the plant and placed directly on the diet in petri dishes. Several larvae could be placed in each dish, since they are not cannibalistic. Field-collected pupae and prepupae were also placed on the diet to prevent their dehydration.

Field-collected individuals were placed in a laboratory incubator under a 24-h dark period at 25±1 C and 70% RH. Larvae were supplied with a fresh diet every week or as needed until pupation or death. Field-collected pupae were placed in an incubator for 24 h at 25± 1 C and 70% RH and weighed and then transferred to 30 ml clear plastic cups. Each cup was wrapped with wet tissue paper and placed in a liter wax-lined ice cream container.

After eclosion, the adults were transferred to oviposition cages made from 4 liter wide-mouthed plastic containers lined with damp paper towels. Organdy cloth was draped over the edge of each container, and allowed to hang down to the bottom. Several waterhyacinth leaves were placed inside each container. For mating, usually two males and one female were placed in each container and the containers were covered with organdy cloth and secured with a rubber band. The oviposition containers were kept separated and placed in locations at room temperature where natural crepuscular light was available. Because adults have vestigial mouthparts, they were not supplied with food.

Eggs were removed from the organdy cloth and waterhyacinth leaves by gently dislodging them between thumb and forefinger. Eggs were counted, sterilized in a 20% formaldehyde solution (37% actual) for 2 min and rinsed in distilled water for 5 min. Twenty sterilized eggs were transferred directly to each diet cup under the microbiological hood and capped with a wax-coated paper lid. The lids prevented the escape of first instar larvae that eclosed in 5 to 6 days.

Larvae were changed 3 times to fresh diet in petri dishes after the 3rd, 5th and 7th molt, as determined by counting the number of head capsules. At the 5th instar change, 5 larvae were placed in each petri dish until development to pupae (Figure 3). Pupae from laboratory rearings were weighed according to the methods described earlier for the field-collected pupae.

RESULTS AND DISCUSSION

The development of artificial diets for rearing phytophagous insects has progressed rapidly through the years (18). In particular, a diet was developed for mass-rearing *Bactra verutana* Zeller (Lepidoptera: Tortricidae) (7), a native biological control agent against purple and yellow nutsedge. Freeze-dried plant parts were first used in the diet but further testing demonstrated that *B. verutana* could be reared on the basic diet without the plant material. This study with diets indicated that *A. densa* needed the freeze-dried waterhyacinth; otherwise, deformations and other abnormalities occurred.

In a previous study on the life history of *A. densa* (23), first instar larvae were started on plant tissue and the second instar larvae were transferred to a sugarcane borer diet (10). Although some larvae completed development, there was no mention of percent survival or possible successive laboratory generations in this study (23).

The artificial diet (Table 1) described herein was used to rear *Arzama densa* from egg to adult through three generations. Although a total of four diet changes were necessary, it is probable that a greater amount of diet per container, combined with flash-sterilizing methods could reduce the number of diet changes.

Life history data on field-collected and three consecutive laboratory-reared generations on artificial diet is summarized in Table 2. The sex ratio favored the male after successive laboratory generations. Developmental time of individuals reared on fresh plant material averaged 56 days (4) and 65 days on the combined plant material and sugarcane borer diet (23). In comparison with this study, the rate of development was longer, averaging 78 days for the three diet-reared laboratory generations. Males completed development several days earlier than the females. Mean developmental time and percent survival (oviposition of egg to adult) could not be determined for the field-collected generation because the individuals were acquired as larvae. Mean developmental times of the three laboratory generations showed a successive decrease.

Fecundity data for field-collected and laboratory-reared females are shown in Table 3. About 75% of the total oviposition occurred on the 1st and 2nd nights of mating. Field-collected females averaged 297 eggs. A slight decrease, to 225 eggs/female was noted in the laboratory F_1 generation, but by the F_3 generation, oviposition approached that of field-collected individuals with 268 eggs/female. Egg

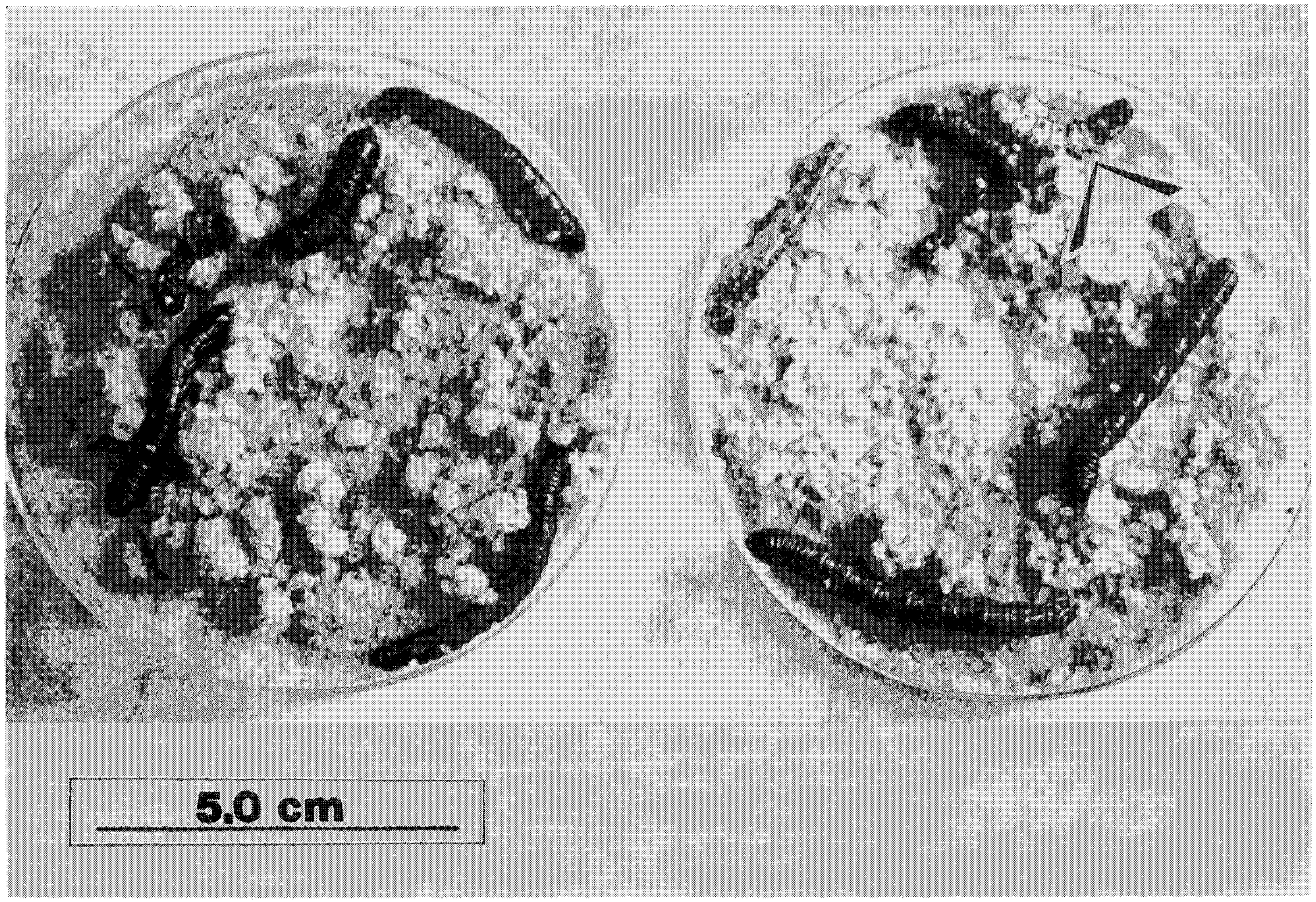

Figure 3. *Arzama densa* 7th instar larvae, prepupa (arrow) and pupae on artificial diet.

TABLE 2. LIFE HISTORY DATA FOR *A. densa* REARED ON ARTIFICIAL DIET.

Generation	Larvae tested	Sex Ratio	Mean developmental time (egg to adult)	Percent survival (egg to adult)
	(no.)	(♂:♀)	(days)	(%)
Field-collected	72	1.2:1	—[a]	—[a]
F_1	150	1.3:1	83(67-112)	69.4
F_2	150	1.3:1	79(64-101)	62.1
F_3	150	1.5:1	72(39-99)	63.2

[a]Individuals acquired as larvae.

TABLE 3. FECUNDITY DATA FROM FIELD-COLLECTED AND LABORATORY-REARED *A. densa*.

Generation	♀♀	Total eggs	$\overline{X}$ eggs/♀	Percent of eggs eclosed
	(no.)	(no.)	(no.)	(%)
Field-collected	28	8,314	297	95.2
F_1	21	4,736	225	97.3
F_2	181	42,061	232	93.5
F_3	200	53,643	268	94.8

eclosion rate averaged 95% in the three laboratory generations.

Timing the emergence of adults for mating was critical because they only live for 4-6 days. Males seemed to lose their vigor 3 days after emergence. The females became "egg bound" or are unable to lay eggs 2 days after emergence. Adult longevity could be extended for an extra day by exposing them to continuous light.

Pupal weights of the field-collected and F_1-F_3 laboratory generations are presented in Table 4. This study represented a method to determine if diet-fed larvae differed from field-collected, plant-fed individuals. Duncan's multiple range test was used to compare differences among pupal weight means. There was a significant increase in weight from 397 to 458 mg of the females of the F_1 generation. However, all other single sex comparisons were not significantly different, indicating no significant decrease between any of the comparisons. These results indicate that diet-fed individuals were at least as large as those collected in the field.

Biotic regulating factors were observed to play an important role in *A. densa* populations. While collecting *A. densa* in the field for initial laboratory colonies, several of these "factors" were noted. Most of the mortality was a result of larval parasitism by *Campoletis oxylus* (Cresson) (Hy-

TABLE 4. MEAN PUPAL WEIGHTS OF 15 INDIVIDUALS OF EACH SEX FROM FIELD-COLLECTED AND F_1-F_3 LABORATORY-REARED GENERATIONS.[c]

	Field-collected	F_1	F_2	F_3
	mg(±SD)	mg(±SD)	mg(±SD)	mg(±SD)
Male	291[b](±56)	331[b](±74)	312[b](±56)	310[b](±68)
Female	397[b](±59)	458[a](±98)	374[b](±76)	382[b](±84)
Both sexes	344[b](±80)	395[a](±107)	343[b](±73)	346[b](±84)

[a,b]Same letter indicates no significant difference, ($\alpha \leq .05$).
[c]Pupae held in an incubator for 24 h at 25 ± 1 C and 70% RH before weighing.

menoptera: Ichneumonidae) that emerged from 4th instar larvae and by *Lydella radicis* (Townsend) (Diptera: Tachinidae) from 7th instar larvae. Preliminary investigations on these two parasites show variations in their population densities during different times of the year with respect to *A. densa* populations.

This study indicates that a native biological control agent against waterhyacinth can be mass-reared as was *Bactra verutana* against two species of nutsedges (7). The moth, *B. verutana*, has been mass-reared to augment natural populations in field research studies (6). *Arzama densa* has shown indications as a potential native biological control agent. Current investigations include developing schedules and determining quantities of eggs and/or larvae needed for field release(s). If utilized correctly, *A. densa* may serve as an example of manipulating one of our native biological control agents. In addition, the moth may serve as a potential classical biological control agent in other countries where waterhyacinth is a growing problem.

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LITERATURE CITED

1. Batanouny, K. H. and El-Fiky, A. M. 1975. The waterhyacinth (*Eichhornia crassipes* Solms) in the Nile System, Egypt. Aquatic Bot. 1:243-252.
2. Bock, J. H. 1966. An ecological study of *Eichhornia crassipes* with special emphasis on its reproductive biology. Ph.D. Dissertation in Botany, University of California. 175 pp.
3. Center, T. D. 1975. The use of insects for the biological control of waterhyacinth in the United States. Pages 51-59 *in* P. L. Brezonik and J. L. Fox (eds.) Proc. Symp. Water Qual. Manage. Through Biol. Contr., Gainesville, Florida, Jan., 1975, EPA Report No. ENV-07-71-1, 164 pp.
4. Center, T. D. 1976. The potential of *Arzama densa* (Lepidoptera: Noctuidae) for the control of waterhyacinth with special reference to the ecology of waterhyacinth [*Eichhornia crassipes* (Mart.) Solms]. Ph.D. Dissertation, University of Florida, 334 pp.
5. Frick, K. E. and P. C. Quimby, Jr. 1977. Biocontrol of purple nutsedge by *Bactra verutana* Zeller in a greenhouse. Weed Sci. 25:13-17.
6. Frick, K. E. and J. M. Chandler. 1978. Augmenting the moth (*Bactra verutana*) in field plots for early-season suppression of purple nutsedge (*Cyperus rotundus*). Weed Sci. 26:703-710.
7. Garcia, C. and K. E. Frick. 1975. *Bactra verutana*, a possible biological control agent of purple and yellow nutsedge: large scale rearing on artificial diet. Ann. Entomol. Soc. Amer. 68:15-18.
8. Gusio, F. J., T. R. Bartley and A. N. Beck. 1965. Water resources problems generated by obnoxious plants. J. Waterways Harbor Div. Amer. Soc. Civ. Eng. 10:47-60.
9. Henneberry, T. J. and Kishaba, A. N. 1966. Cabbage loopers. Pages 461-478 *in* C. N. Smith, Insect Colonization and Mass Production, Academic Press, New York. 618 pp.
10. Hensley, S. D. and A. M. Hammond, Jr. 1968. Laboratory techniques for rearing the sugarcane borer on an artificial diet. J. Econ. Entomol. 61:1742-1743.
11. Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. 347. 32 pp.
12. Holm, L. G. 1969. Weed problems in developing countries. Weed Sci. 17:113-118.
13. Holm, L. G., D. L. Plucknett, J. V. Pancho, and J. P. Herberger. 1977. The world's worst weeds: distribution and biology. The University Press of Hawaii. 609 pp.
14. Ignoffo, C. M. 1963. A successful technique for mass-rearing cabbage loopers on a semisynthetic diet. Ann. Entomol. Soc. Amer. 56:178-182.
15. Penfound, W. T. and T. T. Earle. 1948. The biology of the waterhyacinth. Ecol. Monogr. 18:447-472.
16. Robson, T. O. 1974. Mechanical control. Pages 72-84 *in* D. S. Mitchell, ed., Aquatic Vegetation and Its Use and Control, UNESCO, Paris.
17. Sculthorpe, C. D. 1967. The biology of aquatic vascular plants. St. Martens Press, New York. 610 pp.
18. Singh, P. 1977. Artificial diets for insects, mites and spiders. IFI/Plenum Data Company, New York. 594 pp.
19. Spencer, N. R. 1973. Insect enemies of aquatic weeds. Pages 39-47 in Proc. 3rd Int. Symp. Biol. Control of Weeds. 140 pp.
20. Spencer, N. R. 1974. Insect enemies of aquatic weeds. PANS 20:444-450.
21. Ultsch, G. R. 1973. The effects of waterhyacinths (*Eichhornia crassipes*) on the microenvironment of aquatic communities. Arch. Hydrobiol. 72:460-473.
22. Vogel, E. and A. D. Oliver, Jr. 1969. Evaluation of *Arzama densa* as an aid in the control of waterhyacinth in Louisiana. J. Econ. Entomol. 62:142-145.
23. Vogel, E. and A. D. Oliver, Jr. 1969. Life history and some factors affecting the population of *Arzama densa* in Louisiana. Ann. Entomol. Soc. Amer. 62:749-752.
24. Walker, F. 1864. List of the specimens of Lepidopterous insects in the collection of the British Museum. Part 32, Suppl. Part 2, Catalogue of the Lepidoptera Heterocera. Seventh Ser. 35 Parts, London.
25. Zeiger, C. F. 1962. Hyacinth obstruction to navigation. Hyacinth Control J. 1:16-17.