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HOST SPECIFICITY AND RISK ASSESSMENT OF HETEROPERREYIA HUBRICHI, A POTENTIAL CLASSICAL BIOLOGICAL CONTROL AGENT OF CHRISTMASBERRY (SCHINUS TEREBINTHIFOLIUS) IN HAWAI'I

Stephen D. Hight

U.S. Forest Service, Pacific Southwest Research Station, Institute of Pacific Islands Forestry and University of Hawai'i, Hawai'i Volcanoes National Park Quarantine Facility, P.O. Box 236, Volcano, HI 96785-0236, U.S.A.

Email: hight@nettally.com

Current Address: U.S.D.A., Agricultural Research Service & Center for Biological Control, Room 310, Perry Paige Building, South, Florida A&M University, Tallahassee, FL 32307, U.S.A.

Abstract. Heteroperreyia hubrichi, a foliage feeding sawfly of Schinus terebinthifolius, was studied to assess its suitability as a classical biological control agent of this invasive weed in Hawai'i. No-choice host-specificity tests were conducted in Hawaiian quarantine on 20 plant species in 10 families. Adult females oviposited on four test species. Females accepted the Hawaiian native *Rhus sandwicensis* as an oviposition host equally as well as the target species. The other three species received dramatically fewer eggs. Neonate iarvae transferred onto test plants successfully developed to pupae on *S. terebinthifolius* (70% survival) and *R. sandwicensis* (1% survival). All other 18 test plant species failed to support larval development. A risk assessment was conducted to quantify the suitability of non-target plants as a host to *H. hubrichi* on the basis of the insects' performance at various stages in its life cycle. Risk to all plant species tested was insignificant except *R. sandwicensis*. Risk to this native plant relative to *S. terebinthifolius* was estimated at 1%. Currently this is too high a risk to request introduction of this insect into the Hawaiian environment. Detailed impact studies in the native range of *S. terebinthifolius* are needed to identify the potential benefit that this insect offers. Also, field studies in South America with potted *R. sandwicensis* would give more reliable analysis of this plants risk from natural populations of *H. hubrichi*.

Key Words: Schinus terebinthifolius, Heteroperreyia hubrichi, Rhus sandwicensis, Brazilian peppertree, Christmasberry, classical biological control, host specificity, risk assessment, non-target impacts.

INTRODUCTION

Schinus terebinthifolius Raddi (Sapindales, Anacardiaceae), locally known as Christmasberry in Hawai'i, or Brazilian peppertree in Florida, is an introduced perennial plant established throughout the Hawaiian Islands (Yoshioka and Markin 1991). This species is native to Argentina, Brazil, and Paraguay (Barkley 1944, 1957) and was brought to Hawai'i as an ornamental before 1900 (Neal 1965). As early as 1928, state foresters began planting this tree in reforestation efforts on state forest reserves throughout four of the main Hawaiian Islands (Skolmen 1979). The plant is a dioecious, evergreen large shrub to small tree that has compound shiny leaves. Flowers of both male and female trees are white and the female plant is a prolific producer of bright red fruits. The green foliage and bright red fruits have been popular in Hawai'i for Christmas wreaths and decorations (Wagner *et al.* 1990). A less common Hawaiian name for this plant is "wile-laiki", named for Willie Rice, a politician who often wore a hat lei made of the fruits (Neal 1965).

In Hawai'i, *S. terebinthifolius* has become an aggressive, rapidly spreading weed that displaces native vegetation (Bennett *et al.* 1990, Cuddihy and Stone 1990). The plant

occurs from near sea level to about 920 m (Wagner *et al.* 1990). As early as the 1940's, *S. terebinthifolius* was recognized as an important invader of dry slopes on Oahu (Egler 1942). Hawai'i Department of Agriculture recognizes the plant as a noxious weed (Morton 1978). Conservation organizations consider Christmasberry a high priority target in Hawai'i because it is already widespread and has great potential to increase its range even farther (Randall 1993). The U.S. Fish and Wildlife Service (1998) identified *S. terebinthifolius* as one of the most significant non-indigenous species currently threatening federally listed threatened and endangered native plants throughout the Hawaiian Islands.

Naturalization of *S. terebinthifolius* has occurred in over 20 countries worldwide throughout subtropical (15-30°) areas (Ewel *et al.* 1982). Attributes of the plant that contribute to its invasiveness include a large number of fruits produced per female plant, an effective mechanism of dispersal by birds (Panetta and McKee 1997), tolerance to shade (Ewel 1978), fire (Doren *et al.* 1991), and drought (Nilsen and Muller 1980), and an apparent allelochemical effect on neighboring plants (Medal *et al.* 1999).

As a member of the Anacardiaceae, *S. terebinthifolius* shares its allergen causing properties with other members of the family. While not affecting as many people as some of the more notable members of the Anacardiaceae (poison ivy, poison oak, and poison sumac), the plant sap can cause dermatitis and edema to sensitive people (Morton 1978). Resin in the bark, leaves, and fruit have been toxic to humans, mammals, and birds (Ferriter 1997, Morton 1978). The lumber industry has deemed this plant of little value due to its relatively low quality, its poor form due to the multiple, low stems, and the poisonous, resin byproducts (Morton 1978). The sawdust and smoke are particularly dangerous to sensitized people.

No control method is currently available against large, dense populations of *S. terebinthifolius*. Mechanical removal with heavy equipment or chainsaws can be acceptable along accessible areas, such as ditch banks, utility rights-of-ways, or other disturbed areas (Ferriter 1997). Several herbicides and application methods are available that aid in the control of *S. terebinthifolius* (Ewel *et al.* 1982, Gioeli and Langeland 1997, Laroche and Baker 1994, Woodall 1982). However, these non-biological methods are labor intensive, expensive, and provide only temporary control due to the plant's regenerative capability (Medal *et al.* 1999). In addition, mechanical and chemical controls are unsuitable over a large scale and in most natural settings because they are detrimental to non-target organisms. The plant is intolerant of heavy shading and has been know to die out under some plants, e.g., *Schefflera actinophylla* (Endl.) Harms (Apiales, Araliaceae) (C. Smith, personal communication).

Classical biological control against Christmasberry was initiated in Hawai'i in the mid-1900's (Yoshioka and Markin 1991). Surveys were conducted in South America (primarily Brazil) for potential biological control agents (Krauss 1962, 1963). Three insect species native to Brazil were released into Hawai'i: a seed-feeding beetle, *Lithraeus (=Bruchus) atronotatus* Pic (Coleoptera, Bruchidae), in 1960 (Davis 1961, Krauss 1963); a leaf-rolling moth, *Episimus utilis* Zimmerman (Lepidoptera, Olethreutidae), in 1954-1956 (Beardsley 1959, Davis 1959, Krauss 1963); and a stem-galling moth, *Crasimorpha infuscata* Hodges (Lepidoptera, Gelechiidae), in 1961-1962 (Davis and Krauss 1962, Krauss 1963). The first two species became established but were reported to cause only minor damage (Clausen 1978, Yoshioka and Markin 1991). A seed-feeding wasp, *Megastigmus transvaalensis* (Hussey) (Hymenoptera, Torymidae), accidentally introduced from South Africa, has been found attacking seeds of Christmasberry in Hawai'i since early 1970's (Beardsley 1971, Yoshioka and Markin 1991).

Recent classical biological control efforts against *S. terebinthifolius* have been focused in Florida since the late 1980's. This plant is listed as a Florida noxious weed (FDACS 1994); it is displacing native vegetation in parks and natural areas (Bennett and

Habeck, 1991) and is estimated to infest over 4050 km² (Habeck 1995). Exploratory surveys for natural enemies in Brazil identified at least 200 species of arthropods associated with *S. terebinthifolius* (Bennett *et al.* 1990, Bennett and Habeck 1991, Medal *et al.* 1999). Based on field observations of their damage and lack of records that indicate an association with cultivated plants in Brazil, several insects were selected as biological control candidates for further study in Florida. Host specificity studies were conducted on the sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae) in Brazil and at a Florida quarantine facility (Medal *et al.* 1999). Larval development and female oviposition tests of *H. hubrichi* were conducted on 36 plant species in 15 families. The insect was determined to be host specific to *S. terebinthifolius* and a request for release of this insect into the Florida environment is currently under evaluation by Animal and Plant Health Inspection Service, USDA (Medal *et al.* 1999).

Capitalizing on biological studies and host specificity tests conducted in Brazil and Florida, a biological control project was initiated to evaluate the potential of *H. hubrichi* as a control agent of *S. terebinthifolius* in Hawai'i (Hight *et al.* in press). This paper presents a synopsis of the investigation on the host range of *H. hubrichi* in Hawaiian quarantine and a risk assessment for non-target plants.

MATERIALS AND METHODS

Twenty plant species underwent host specificity testing in the Volcano Quarantine Facility. The selected plants belonged to one of three groupings: taxonomically associated plants, habitat associated native plants, and habitat associated agricultural plants (Table 1). Plant relatedness is based on the phylogenetic system of Cronquist (1981). The order Sapindales has 15 families and four of these families (Anacardiaceae, Rutaceae, Sapindaceae, and Zygophyllaceae) have native as well as introduced members in Hawai'i. The single, native, Hawaiian species of Zygophyllaceae, Tribulus cistoides L., was not tested because it occurs only in coastal habitats below 50 m elevation (Wagner et al. 1990). Of the remaining 11 families, only members of the family Meliaceae have been introduced into Hawai'i. Plants that make up the second group are native plants that occur in the same habitat and are therefore likely to be exposed to any introduced biological control agent. The second group is not as closely related to S. terehinthifolius, although members in three families (Araliaceae/Apiales, Myrtaceae/Myrtales, and Fabaceae/Fabales) are in the same subclass (Rosidae). The third group contains two important, woody, crops that are found associated with S. terebinthifolius habitat. These two species are in the same subclass as S. terebinthifolius.

Insect Material.

Two shipments of *H. hubrichi* were imported from Brazil into the Hawai'i Volcanoes National Park Quarantine Facility. The first shipment was received 19 November 1998 and consisted of 236 neonate larvae, which eclosed from four egg masses, and 192 late instar larvae. The second shipment arrived 22 March 1999 and contained 101 late instar larvae. Individuals of both shipments were collected in southern Brazil around the city of Curitiba, Paraná State. Quarantine host specificity tests were conducted from subsequent generations reared in captivity.

Both male and female adults can fly, although the male is a stronger flyer. Neither the male or female adult *H. hubrichi* feed. However, both sexes were observed drinking from small water droplets.

Adult Oviposition Tests.

No-choice oviposition tests were conducted in the quarantine facility on cut shoots for each of the 20 test plant species. Tests were conducted in plastic containers holding a single stem of a test plant (with 2 to 4 leaves). A mated female *H. hubrichi* was placed on the test plant and if she oviposited, she remained inside the container with her eggs. If a female did not oviposit on the test plant within 48 to 60 hr, she was removed and placed in a new oviposition arena with a stem of *S. terebinthifolius* to evaluate her fecundity. Number of eggs laid and viability of eggs were recorded. For all plant species, tests were replicated at least four times. *Citrus sinensis* (L.) Osbeck (Sapindales, Rutaceae) was not tested because of lack of plant material. Tests on this plant in Florida and Brazil found this to be an unacceptable host plant.

Oviposition tests were conducted on potted plants of five test species on which oviposition has occurred and/or on which larvae had developed on cut shoots. A mated female was placed on the caged test plant until she died. The number of eggs laid and viability of eggs was recorded. Each plant species was replicated at least six times.

No-Choice Larval Development Tests.

All test plants were evaluated as to their ability to support larval development under nochoice conditions. Unfed, neonate larvae less than two hours old were transferred to small cut shoots of the test plant stuck into moistened florist-foam-filled vials and reared in 480 ml plastic containers. Since larvae feed gregariously, 15 larvae were transferred into each container with a fine tip brush. Each test plant was replicated at least six times. For each family of larvae used in the tests, 2-3 replicates of 10-15 larvae were reared on *S. terebinthifolius* to insure the vitality of each egg mass. Containers were cleaned, larvae were fed, and mortality was assessed on the third day after transfer and then every fourth day. Containers were evaluated every day after larvae became sixth instars.

Larval development tests were also conducted on potted plants of five test species because of oviposition activity and/or larval development on cut shoots. Each plant had an egg mass of *H. hubrichi* either naturally oviposited on the stem or tied onto a stem from a successful oviposition on *S. terebinthifolius*. The number of larvae that successfully developed on each test plant was recorded. The test was replicated on each plant species at least three times.

Relative Host Suitability

In an attempt to quantify potential suitability of non-target plant species for agent development, Wan and Harris (1997) developed a scoring system that compares the suitability of non-target species to that of the target species. I have followed their approach to obtain estimate host suitability. The index of suitability of a non-target host plant for *H. hubrichi* use is $R_1 \times R_2 \times ... R_n$, where *R* is the performance of the insect at various life stages on the test plant relative to that on *S. terebinthifolius*. Suitability parameters estimated for each test plant species included the proportion of females that oviposited on the plant, number of eggs oviposited, proportion of larvae that survived, and development time of larvae from eggs to pupae (Table 2). For purposes of calculation, zero values for any parameter (complete rejection or failure) were taken to be 0.001.

RESULTS

Insect Biology.

The adults of *H. hubrichi* are generally black with yellow legs. A female and male *H. hubrichi* mate on the surface of soil or plants, although females do not need to mate for oviposition to occur. Each female oviposits her eggs in a single mass just into the surface of non-woody stems. Eggs in a mass are arranged in rows and the female

"guards" her eggs until she dies, just before the eggs hatch. Eggs hatch in 14 days. Neonate larvae feed gregariously on both surfaces of young leaflets at the tip of shoots. As they grow they move as a group onto new leaflets and larger leaves until the third to fourth instar when they disperse throughout the plant and feed individually. A larva is green with red spots and black legs. After reaching the seventh instar, the larva moves into soil and pupates. Insects reared on *S. terebinthifolius* took 26-42 days from egg hatch to pupation. Pupation lasted two months for 80% of pupae and the longest successful pupation occurred in seven months.

Adult Oviposition Tests.

Female *H. hubrichi* oviposited on cut shoots of five different test plant species (Table 1). All females that were placed on *S. terebinthifolius* and *R. sandwicensis* oviposited on their test plant. Less than half of the females placed on the other four test species successfully oviposited on their test plant (Table 2). However, all non-ovipositing females successfully oviposited once they were moved onto *S. terebinthifolius* after the 48-60 hr test period. This indicated that the females were capable of ovipositing on the test plant but rejected that plant species as an oviposition host.

Mean number of eggs oviposited by females on each test plant species is presented in Table 1. There was no significant difference in the number of eggs deposited on *R.* sandwicensis and on *S. terebinthifolius* (*t*-test; p > 0.05; $t_{(43)} = 1.762$). Oviposition on the three Sapindaceae test plant species was highly variable with most tests receiving no eggs. In those plants receiving eggs, the average number deposited was high: Dodonaea viscosa – 57 eggs; Litchi chinensis – 78 eggs; and Euphoria longan – 56 eggs.

Oviposition was more restricted on potted plants than on cut shoots. Mated *H. hubrichi* females oviposited on only three of the five species of potted test plants (*S. terebinthifolius*, *R. sandwicensis*, and *E. longan*). Females did not oviposit on potted *D. viscosa* or *L. chinensis*, even though oviposition did occur on cut shoots of *D. viscosa* and *L. chinensis*.

No-Choice Larval Transfer Tests.

Neonate larvae successfully developed on cut shoots of only two test plant species, *S. terebinthifolius* and *R. sandwicensis*. Larvae on most of the other test plant species were dead within seven days (Table 1). Although cut shoots of two additional plant species supported some larval development for more than two weeks, (*Mangifera indica* (Sapindales, Anacardiaceae), 23 d and *E. longan* (order, family) 19 d), no larvae survived to pupation.

Successful larval development on the five potted plant species was similar to development on cut shoots. Larvae developed only on potted *S. terebinthifolius* and *R. sandwicensis*. The proportion of larvae successfully developing on *S. terebinthifolius* and *R. sandwicensis* potted plants was slightly higher to the proportion on cut shoots of those two test plant species (78% and 4%, respectively).

Relative Host Suitability

The relative host suitability of the test plant species is shown in Table 3. Suitability estimates are calculated only for the five plant species that received eggs from ovipositing females. Scores for all four non-target plants were lower than for *S. terebinthifolius*, measured at 1.0. All other 15 tested species were unacceptable host for both oviposition and larval development and are not at risk by the release of *H. hubrichi* into Hawai'i.

DISCUSSION

Field observations in Brazil and laboratory feeding tests in Florida indicated that H. hubrichi was highly host specific and safe to release into the Florida environment (Medal et al. 1999). Additional host specificity studies in guarantine primarily on native Hawaiian plants confirmed a highly limited host range for *H. hubrichi*. Tests at all locations showed that S. terebinthifolius was the preferred, if not the only host plant of H. hubrichi. However, the potential host range in Hawai'i appears to be slightly broader than that identified in Florida and Brazil. Tests in Florida evaluated two North American species of sumac (*R. copallina* and *R. michauxii*) and found them unsuitable for *H. hubrichi* oviposition and incapable of supporting larval development (Medal et.al. 1999, J. Cuda, personal communication). Hawaiian tests indicated that the Hawaiian sumac (R. sandwicensis) did support larval development and was highly attractive to the female for oviposition. Chemicals still present in ancestral, continental species that deter herbivorous insects may have been lost over time in the Hawaiian sumac. Of the five varieties of S. terebinthifolius recognized in South America (Barkley 1944), H. hubrichi prefers the most pubescent variety (M. Vitorino, personal communication). The dense pubescent nature of R. sandwicensis may stimulate female oviposition regardless of the quality of the plant for larval development. Both S. terebinthifolius and R. sandwicensis were comparable in their acceptance by ovipositing females as measured by proportion of females that oviposited on the test plant and the number of eggs that a female laid. But R. sandwicensis was a dramatically poor host for H. hubrichi larvae in both performance characteristics of larval survival and development time.

To identify the potential non-target effect that native *R. sandwicensis* might be exposed to because of the introduction and release of *H. hubrichi* into Hawai'i a host suitability assessment was conducted. To arrive at realistic estimates of host suitability, both physiological and behavioral processes must be estimated (McEvoy 1996). Estimates of host suitability were determined by quantifying crucial stages in the sawflies sequence to locate, accept, and develop on the host, i.e., oviposition by the female, larval development time, and larvae survival rate from egg to pupa in no-choice tests.

Relative host suitability of non-target species was evaluated for the five test plant species that experienced any establishment and/or damage from *H. hubrichi* in the host specificity tests. Four plant species had extremely low levels of suitability (Table 3). In fact, since all four of these plants completely failed to support larval development it may be argued that their suitability for *H. hubrichi* development is zero. The life cycle of *H. hubrichi* would be interrupted if the insects were to colonize any one of these plants and a population of *H. hubrichi* would fail to establish. A low suitability level was measured for *R. sandwicensis* (approximately 1%).

Introduction of *H. hubrichi* into Hawai'i will not be requested at this time because of the apparent risk to *R. sandwicensis*. However, additional information is being sought which may reverse this decision. Field experiments in Brazil with potted *R. sandwicensis* are being proposed to evaluate the risk of this non-target plant under more natural settings. The observed host range of herbivorous insects is often wider under laboratory-based tests than open-field tests (Cullen 1990, Briese 1999). In addition, the risk inherent in introducing a biological control agent may be outweighed by its benefit. Therefore, detailed impact studies are needed in Brazil to evaluate the effect *H. hubrichi* has on *S. terebinthifolius* fitness. Neither of these types of tests is currently funded.

Additional surveys for phytophagous insects of *S. terebinthifolius* should be conducted in northern Argentina, the most likely center of origin of this species (Barkley 1944). Virtually all previous South American explorations by scientists from Hawai'i (Krauss 1962, 1963) and Florida (Bennett *et al.* 1990, Bennett and Habeck 1991) have

taken place in southern Brazil. Although this work has identified several promising biological control candidates, additional surveys may be more successful in Argentina. For example, on a 10-day survey in January 2000 of *S. terebinthifolius* natural enemies in the state of Missiones, Argentina, two species of stem boring Cerembicidae and a bark girdling Buprestidae were collected (S. Hight, unpublished data). Identifications of these insect species are pending. No stem boring or bark girdling insects were identified from Brazilian surveys.

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Table 1. Host specificity tests on cut shoots for the Brazilian schinus-sawfly *Heteroperreyia hubrichi* in Hawaiian quarantine.

		Reps. : No. individuals ²	ndividuals ²	No. eggs	Larval	Avg. lan	Avg. larval survival (%)	(%) le
Plant family	Plant species ¹	Oviposition	Larval dev.	oviposited (mean ± SE)	feeding ³	3 days	7 days	Final
A. Taxonomically	A. Taxonomically Associated Plants	S						
Anacardiaceae	Schinus terebinthifolius	35	87 : 1032	115 ± 23.0	+ + +	94	88	70
	Rhus sandwicensis*	11	24:401	118 ± 20.7	+ +	72	24	-
	Mangifera indica	Q	6 : 120	0	(+)	97	92	0
Sapindaceae	Dodonaea viscosa*	24	6 : 90	5±21.2	1	2	o	o
	Sapindus saponaria⁺	4	06 : 9	0		ω	o	0
	Litchi chinensis	6	6 : 90	34 ± 49.8	(+)	32	0	o

	Euphoria Iongan	12	10:159	23 ± 36.6	(+)	53	17	0
	Alectryon subcinereum	5	6 : 90	0	ł	8	8	0
	Nephelium mutabile	6	8 : 120	0	B	12	0	0
Rutaceae	Melicope hawaiensis*	6	6 : 90	0	1	37	0	0
	Citrus sinensis	0	7 : 105	0	1	57	0	0
 B. Habitat Associated Native Plants 	ciated Native							
Araliaceae	Reynoldsia sandwicensis*	5	6 : 91	0	I	41	0	0
Myrtaceae	Metrosideros polymorpha*	4	7 : 104	0	1	62	0	0
Fabaceae	Acacia koa*	4	12:180	0	I	2	0	0
	Sophora chrysophylla*	4	12 : 180	0	1	17	Q	0
Myoporaceae	Myoporum sandwicense*	4	6 : 90	0	I	39	0	0

Convolvulaceae	Ipomoea indica*	Q	6 : 90	0	ł	37	0	0
Dicksoniaceae	Cibotíum glaucum*	4	6 : 90	0	I	16	0	0
C. Habitat Associated Agricultural Plants	iated							
Proteaceae	Macadamia integrifolia	4	6 : 90	0	1	29	0	o
Rubiaceae	Coffea arabica	4	6 : 90	o	t	4	0	o

¹ * = Species native to Hawai'i

² Number of individuals tested for oviposition is same as number of oviposition tests, but number of individuals tested for larval

development varied with test. ³ + + + indicates normal larval feeding; + + indicates moderate larval feeding; + indicates slight larval feeding; (+) indicates occasional nibbling by larvae; and – indicates that no larval feeding occurred.

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hubrichi on various host plant test species in Hawaiian quarantine.	
nce of Heteroperreyia hubn	
Table 2. Performar	

Component of			Host Plar	Host Plant Test Species	se	
Host Usage for Suitability Assessment	Performance Measure	Schinus terebinthifolius	Rhus sandwicensis	Dodonaea viscosa	Litchi chinensis	Eu[hproa longan
Establishment	 proportion of females that oviposit 	1.000	1.000	0.083	0.438	0.417
Establishment	 mean number of eggs oviposited 	115	118	5	34	23
Establishment	 mean development time of larvae (days) 	36.547	47.000	0	ο	0
Damage	 proportion of eggs that survive to pupae 	0.697	0.008	0	o	o

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Table 3. Relative host suitability analysis for *Heteroperreyia hubrichi* performance on various host plant test species in Hawaiian quarantine.

		Performan	ce Measure ¹	,	
Plant Species	1	2	3	4	Relative Suitability
S. terebinthifolius	1.000	1.000	1.000	1.000	1.000
				_	
R. sandwicensis	1.000	1.026	0.778	0.011	0.009
D. viscosa	0.038	0.043	0.001	0.001	3.6 x 10 ⁻⁹
L. chinensis	0.438	0.296	0.001	0.001	1.3 x 10 ⁻⁷
E. longan	0.417	0.200	0.001	0.001	8.3 x 10 ⁻⁸

¹ Performance measure estimates are relative to *S. terebinthifolius.* 1 = proportion of females that oviposit; 2 = mean number of eggs oviposited; 3 = mean development time of larvae; 4 = proportion of eggs that survive to pupae.