Discrimination by Ovipositing Boll Weevils (Coleoptera: Curculionidae) Against Previously Infested *Hampea* (Malvaceae) Flower Buds

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ABSTRACT The low occurrence of multiple oviposition punctures in flower buds of *Hampea nutricia* indicated an avoidance of already infested buds by female boll weevils. This behavior was confirmed by observations which showed that individual weevils inspected buds before oviposition and rejected them if a puncture plugged with frass was encountered. Free-choice tests showed that females preferred uninfested buds for oviposition over either infested buds or buds implanted with a frass plug taken from an infested bud. Forcing weevils to oviposit in infested buds greatly decreased egg production.

EARLY IN the century, it was observed that uninfested cotton flower buds were selected by boll weevils for oviposition in preference to infested buds, and generally only one egg was deposited per bud except in no-choice situations (Hunter and Pierce 1912). In spite of this interesting observation, little subsequent work has been done to determine the mechanism underlying this behavior. Among the studies conducted to date, conclusions have been contradictory. Everett and Earle (1964) suggested that secretions, possibly originating from the collateral gland, are used by the weevil to seal oviposition punctures and could function to deter other females from ovipositing. In a similar vein, Hedin et al. (1974) mention a suggestion made by W. H. Cross that marking pheromone(s) which act as oviposition deterrents might occur in puncture-sealing frass. Mitchell and Cross (1969) described oviposition behavior of the boll weevil, including inspection of flower buds, and an apparent ability to perceive previous punctures. Jenkins et al. (1975) later cast doubt on the existence of such a deterrent based on the frequency distribution of egg punctures on cotton buds from fields in Mississippi. McKibben et al. (1982) attempted to resolve this contradiction by hypothesizing that exposure to infested buds raises the response threshold. These authors showed that a computer simulation of this model generated a distribution of ovipositions which was indistinguishable from random except at high infestation levels.

In the face of the uncertainty concerning this weevil's ability to respond to infested buds, it seemed useful to consider oviposition behavior in a population of boll weevils assumed to be isolated from, and unaffected by, any changes that have occurred in the last 80 years in the weevils of the United States. One such population occurs in southeastern Mexico where the host plant is an abundant and fast-growing native tree, *Hampea nutricia* Fryxell (Malvaceae). There we carried out the observations and experiments described below with the object of answering two related questions: (1) Is the distribution of oviposition punctures found in the field independent of previous ovipositions, or does it indicate discrimination by weevils against punctured buds, and (2) Can discrimination behavior be demonstrated with individual weevils under laboratory conditions? Preliminary results were strongly in favor of the discrimination hypothesis and we were encouraged in our efforts to determine what stimulated this behavior.

Materials and Methods

Field data on the distribution of oviposition punctures was limited to buds of staminate *H. nutricia* trees. Weevils do not ordinarily oviposit in buds or fruit of pistillate trees of this dioecious species (P. Stansly, unpublished data). Data were collected from 18 August to 16 November 1980 at three sites, each ca. 20 km in different directions from Cardenas, Tabasco, Mexico. Sixteen trees from 3 to 8 m in height were sampled at weekly intervals by cutting branches at random with pruning shears or a tree-trimmer. Buds from cut branches were separated in the laboratory and scored for weevil punctures. Damaged plant material was dissected to detect whether or not immature weevils were present.

Preference tests and behavioral observations were carried out in a laboratory at the Colegio Superior de Agricultura Tropical. Weevils were collected in the field as adults ($n = 3$) or as larvae and reared to adults in the laboratory ($n = 16$). Adults were maintained separately on a 13L:11D photoperiod at an average of 30.4°C (SD = 1.6, range, 25.5–34.1°C) and 80% relative humidity (SD = 6.3, range, 65–92%) as measured by a mechanical hydrothermograph. Each weevil was fed

daily by placing freshly cut branches of *Hampea* containing undamaged buds in 500-ml Erlenmeyer flasks filled with water and covered with a sleeve of nylon marquisette 10 cm in diameter and 60 cm long. The sleeve was taped to the bottom of the flask and tied at the top above the branch. Egg production was recorded daily. Choice tests lasting 24 h were performed in the same cages by substituting branches containing buds of predetermined number and type for the normal food ration. Infested buds were produced when needed by using branches that had been exposed to another weevil the previous day, adjusting the number of punctured and unpunctured buds by removal, and marking both old punctures and fresh buds with india ink. The ratio of uninfested to infested material ranged from 0.15 to 5.7 in these experiments in order to include the infestation level at which some weevils would be forced either to oviposit in infested buds or decrease their normal production. In all other choice tests the number of treated and control buds was kept approximately equal. Number of weevils used, and the tests performed for each comparison, are shown in Table 1.

In additional treatments, frass plugs from infested buds obtained as described above were removed with jeweler's forceps and implanted in a small hole made in the corolla of a fresh bud, where oviposition normally occurs. To test solubility of the active ingredients in a nonpolar solvent, from 25 to 100 frass plugs were removed from buds exposed to weevils the day before and soaked in petroleum ether for from 1 to 5 days before implantation. The extract so obtained was dripped onto fresh buds with a Pasteur pipette. Activity of the treated plugs was also tested. Depending on the treatment, controls consisted of clean buds, buds implanted with fresh plugs, buds punched with a sharp tool, or buds treated with plain petroleum ether.

After each 24-h testing period, branches were taken from the cages and the buds removed and scored according to whether or not new punctures and plugs had appeared. Initially, all newly punctured buds were dissected to verify the presence of an egg. However, as the number of buds to be dissected increased, and it was seen that the presence of a plug almost invariably indicated the presence of an egg (eggs per plug = 0.98, SE = 0.001, $n = 62$), only spot dissections were performed. The use of sealed punctures was also considered by Everett and Ray (1962) to be a reliable indication of oviposition by weevils in cotton. When weevils were forced to oviposit in infested buds, 94% (SE = 0.4, $n = 22$) of the most recently made plugged punctures contained eggs.

Observations on ovipositional behavior were made on three weevils by lowering the marquisette sleeve so that the weevil's movements could be seen. A stopwatch was used to time searching and oviposition.

Results

Field Observations. A total of 3,614 male *Hampea* buds were examined in 1980. Of these 964 had one plugged puncture, 7 had two and none had more than two. If oviposition occurred independently of the presence or absence of a previous puncture, a Poisson model should fit these data without significant deviation. However, the actual distribution varied significantly from that predicted by the Poisson ($\chi^2 = 165.9$, $P < 0.0001$) (Sokal and Rohlf 1981). The largest deviation from the Poisson model occurred in the class of multiple-plugged punctures (expected = 109). The weevils appeared to be avoiding ovipositing in infested buds, providing clear evidence to reject the null hypothesis of nondiscrimination.

There were too few doubly punctured buds to discern any particular pattern in their occurrence. They were found over the entire season and there was little correlation with date ($r = 0.039$) or percentage of buds punctured ($r = 0.12$). Two of the doubly punctured buds contained no weevils and one contained a second-instar larva and an empty cell. The four remaining buds all contained two immature weevils. Two contained either eggs or newly hatched first-instar larvae, one had a second instar and a dead first-instar larva, and one contained a second-instar and a small third-instar larva. Judging from past experience, all these larvae had been developing for ca. 1 or 2 days since hatching.

Behavioral Observations. The ovipositing weevil pierced the corolla and androecium with its mandibles (average time 9.6 min, SE = 0.45, $n = 12$), after which it made a 180° rotation, inserted the ovipositor into the newly excavated hole and deposited an egg (average time 1.3 min, SE = 0.02, $n = 12$). The female then sealed the puncture by defecating into it, tamping down the frass with several rapid vertical motions of the abdomen (Fig. 1). This tamped frass formed a plug approximately 1 mm in diameter which was yellow at first (like the *Hampea* pollen it was seen to contain), and darkened to a reddish brown in a few days. Virtually all oviposition punctures made by weevils in the laboratory were sealed in this way (99.6%, SE = 0.2, $n = 246$).

The weevil rested for a period of 17.5 min (SE = 12, $n = 8$), usually at the base of the peduncle, before resuming activity with a rapid search along the branch until it encountered another flower bud. It then ran over the bud antennating, paying special attention to the corolla. On uninfested buds, average inspection time was 29.5 s (SE = 12, $n = 8$) before a site was selected and the puncture begun. However, on infested buds ($n = 16$), the frass plug was soon encountered, antennated, and even nibbled, after which the weevil moved off the bud to continue searching elsewhere (Fig. 2).

Fig. 1. Female boll weevil, having just finished ovipositing, plugging puncture with frass.

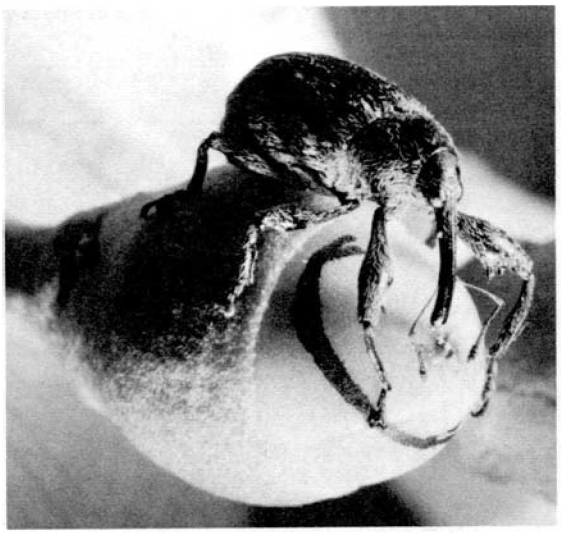

Fig. 2. Searching female boll weevil homing in on frass plug.

Laboratory Experiments. In laboratory studies, there was a highly significant preference for uninfested buds compared to infested buds for oviposition ($\chi^2 = 508$, $P < 0.0001$, Table 1a). These results were highly significant despite the fact that low numbers of uninfested buds in some of the trials forced weevils to oviposit in infested buds.

Clean buds implanted with fresh oviposition plugs were strongly avoided also ($\chi^2 = 122$, $P < 0.0001$, Table 1b), showing that the oviposition plug was sufficient to initiate discriminatory behavior. Relatively fewer "mistakes" were made by the weevils in experiments with implanted buds than in experiments with infested buds because in the former there was always a choice.

The following results are statistically significant though possibly not definitive due to the small number of animals tested. Untreated control buds were preferred over buds implanted with plugs soaked in petroleum ether, but these in turn were preferred over buds implanted with fresh plugs ($\chi^2 = 30.1$, $P < 0.0001$, $\chi^2 = 10.6$, $P < 0.001$, Tables 1 c and d). Thus some deterrent activity was removed by the ether treatment. Treatment of buds with a petroleum ether extract of plugs did not affect choice compared to controls treated with ether alone ($\chi^2 = 0.32$, $P < 0.57$, Table 1e). Therefore, deterrent substances removed by ether were either transformed in some way or were too dilute to cause discrimination.

The data in Table 1(f–j) demonstrate that a puncture alone did not elicit discriminatory behavior, but that deplugging infested buds did not completely remove the avoidance stimulus. Buds punctured with a sharp tool were accepted as readily as unpunctured buds ($\chi^2 = 0.001$, $P = 0.97$, Table 1f) and were preferred both over punctured buds implanted with a plug ($\chi^2 = 37.0$, $P < 0.0001$, Table 1g), and over infested buds with plugs removed ($\chi^2 = 50.1$, $P < 0.0001$, $\chi^2 = 15.5$, $P < 0.0001$, Tables 1 h and i). When offered a choice between buds containing a plug and infested buds with the plug removed, the expected frequencies were too small to use the χ^2 test and a G test was used instead (Sokal and Rohlf 1981). It showed no significant difference between deplugged infested buds and plugged infested buds as oviposition sites ($G = 2.4$, $P < 0.12$, Table 1j). It is not clear if this result was due to the presence of active substances separate from the frass plug, or to contamination with plug material itself.

Virgin females (which seldom oviposited and usually on the outside of the bud) discriminated against infested buds in their feeding, but males did not ($\chi^2 = 39.8$, $P < 0.0001$, $\chi^2 = 2.2$, $P = 0.14$, Table 2). Thus, only females discriminated against infested buds, and they did so whether or not they were ovipositing.

There were data that indicated that the presence of infested buds had a suppressive effect on oviposition. Reproductively active weevils, when provided only with uninfested buds, oviposited an average of 18.8 eggs per day ($n = 85$, SE = 1.1). In contrast, when infested material was also present only 12.9 eggs per day ($n = 63$, SE = 0.8) were laid, which was significantly fewer ($P < 0.001$, Wilcoxon paired ranks test). When the ratio of infested to uninfested was 2:1 or greater, these same weevils oviposited only 10.2 eggs per day ($n = 19$, SE = 1.1). Weevils forced to oviposit in infested buds by the lack of clean material decreased egg production to half or less of former values, in some cases even stopping completely.

Ten doubly infested buds were dissected 8 days after the second oviposition. All 10 buds contained

Table 1. Numbers of *H. nutricia* flower buds accepted and not accepted by female boll weevils in oviposition choice tests; data arranged in contingency tables and analyzed for independence by χ^2

	1a.	Accepted	Not accepted
Uninfested	Obs.	649	500
	Exp.	384	764
Infested	Obs.	183	1,155
	Exp.	448	890

Number of weevils = 9
Number of trials = 76
$\chi^2 = 508$, $P < 0.0001$

	1b.	Accepted	Not accepted
Uninfested	Obs.	119	18
	Exp.	84	53
Plug implanted	Obs.	3	60
	Exp.	38	25

Number of weevils = 2
Number of trials = 5
$\chi^2 = 122$, $P < 0.0001$

	1c.	Accepted	Not accepted
Uninfested	Obs.	56	13
	Exp.	42	27
Ether-soaked plug	Obs.	13	31
	Exp.	7	11

Number of weevils = 2
Number of trials = 3
$\chi^2 = 30.1$, $P < 0.0001$

	1d.	Accepted	Not accepted
Fresh plug	Obs.	1	13
	Exp.	6	20
	Obs.	13	19
Ether-soaked plug	Exp.	8	24

Number of weevils = 1
Number of trials = 2
$\chi^2 = 10.6$, $P = 0.001$

	1e.	Accepted	Not accepted
Plug extract	Obs.	15	14
	Exp.	14	15
Ether	Obs.	17	21
	Exp.	18	20

Number of weevils = 1
Number of trials = 1
$\chi^2 = 0.32$, $P = 0.57$

	1f.	Accepted	Not accepted
Whole buds	Obs.	39	8
	Exp.	39	8
Punched buds	Obs.	20	4
	Exp.	20	4

Number of weevils = 2
Number of trials = 2
$\chi^2 = 0.001$, $P = 0.97$

	1g.	Accepted	Not accepted
Plug implanted	Obs.	0	27
	Exp.	11	16
Bud punched	Obs.	20	4
	Exp.	9	15

Number of weevils = 2
Number of trials = 2
$\chi^2 = 37.0$, $P < 0.0001$

	1h.	Accepted	Not accepted
Uninfested	Obs.	28	1
	Exp.	14	15
Plug removed	Obs.	3	33
	Exp.	17	19

Number of weevils = 2
Number of trials = 2
$\chi^2 = 50.1$, $P < 0.0001$

	1i.	Accepted	Not accepted
Bud punched	Obs.	12	1
	Exp.	7	6
Plug removed	Obs.	3	13
	Exp.	8	8

Number of weevils = 1
Number of trials = 1
$\chi^2 = 15.5$, $P < 0.0001$

	1j.	Accepted	Not accepted
Plug present	Obs.	0	36
	Exp.	1.5	34.5
Plug removed	Obs.	3	33
	Exp.	1.5	34.5

Number of weevils = 2
Number of trials = 2
$G = 2.4$, $P = 0.12$

one weevil, either a large third instar or a pupa. It must be assumed that one of the original weevils, (probably the one younger by a day), was killed and possibly consumed by the other.

Discussion

Both field and laboratory data summarized above leave little doubt that boll weevils on *Hampea* in Tabasco discriminate strongly against infested buds, and that this behavior is responsible for the lower observed number of multiple ovipositions than predicted by a Poisson model. The extreme scarcity of multiply punctured *Hampea* buds in Tabasco is in sharp contrast to the situation in Mississippi cotton, as reported by Jenkins et al. (1975). These authors found 40 and 49% multiply punctured buds in the respective 2 years of their study. How can this difference between two weevil populations considered conspecific (H. R. Burke, personal communication) be accounted for? In the Tabasco system, the small size of *Hampea* buds is probably the reason that no more than one weevil survives in cases of multiple oviposition. The ultimate death of all but the first egg laid must create strong selective pressure to discriminate against an

Table 2. Numbers of *H. nutricia* flower buds accepted and not accepted for feeding by male (a) and virgin female (b) *A. grandis*; data arranged in contingency tables and analyzed for independence by χ^2

	2a.	Accepted	Not accepted		2b.	Accepted	Not accepted
Uninfested	Obs.	14	10	Uninfested	Obs.	31	9
	Exp.	12	14		Exp.	21	32
Infested	Obs.	11	18	Infested	Obs.	11	57
	Exp.	15	17		Exp.	35	55
		Number of males = 3 Number of trials = 2 $\chi^2 = 2.2$, $P = 0.14$				Number of virgin females = 5 Number of trials = 6 $\chi^2 = 39.8$, $P < 0.0001$	

infested bud. Hunter and Pierce (1912) noted that only one weevil emerged from a cotton bud, regardless of the number of ovipositions. In more recent times, it is known that some second ovipositions are successful, particularly in cotton fruit (bolls) and large buds (Hunter et al. 1965). It is common for numerous weevils to emerge from a boll, which may become considerably larger than the bud and still be susceptible to weevils (Hunter and Pierce 1912) whereas *Hampea* fruit is never successfully attacked. In cotton fields, buds are scarce early in the season, while in late season, there are few uninfested buds or bolls. At such times, the advantages of multiple oviposition over no oviposition would select for a less discriminating weevil. Such selection could act by raising the threshold at which the marking pheromone is perceived by or responded to by the weevil. Such a mechanism can explain the differences in discriminatory behavior observed in cotton and *Hampea*. If the reported contrast between modern cotton weevils and those studied at the turn of the century is real, a modern evolutionary trend toward nondiscrimination may be indicated. Even today, differences in degree of discriminatory behavior have been observed between weevil strains on cotton (J. N. Jenkins, personal communication). Such differences may explain some of the discrepancies reported. It should be noted that it is not known whether the weakness or lack of discriminatory behavior in these modern cotton weevils is due to the absence of stimulatory substances or the inability to respond to such substances under certain conditions, as suggested by McKibben et al. (1982).

It is also of more than academic interest to determine the biological origin and chemical nature of the deterrent. Virtually nothing is known about it, except for its moderate insolubility in petroleum ether. The putative substance(s) could either be altered or unaltered constituents of *Hampea* pollen, or synthesized de novo by the weevil. Isolation and identification of the active materials from frass plugs themselves would be an important step in gaining a better understanding of weevil biology, and might also provide a powerful research and management tool.

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