

Oviposition Behaviour of the Woodwasp, *Sirex noctilio* F.

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Abstract

The basic biology of the female woodwasp and factors affecting oviposition behaviour are described. The rate of egg deposition is related to the physiological condition of the host tree; it is demonstrated that the wasp's ability to assess its host tree is directly related to the osmotic quality of the phloem sap.

Introduction

Differences in the general oviposition behaviour of woodwasps was discussed by Wolf (1967) and Morgan (1968), and the ability of *Sirex noctilio* F. to construct single or multiple drills in the xylem through a single hole in the phloem was noted by Coutts (1965). Coutts and Dolezal (1969) reported that when a single drill was made only the arthrospores of the symbiotic fungus, *Amylostereum areolatum* (Fr. Boidin), were deposited, whereas in multiple drills the fungus occurred in one drill and eggs in the other(s). Madden (1968) reported that the tendency of the female woodwasp to make single or multiple drills was related to the physiological status of the drilling site measured in terms of its osmotic pressure.

The mechanics of drilling by *S. noctilio* are essentially as described by Ass and Funtikow (1932) for *Sirex gigas* L. and *Paururus* (*Sirex*) *juvencus* L.; this paper considers the role of both intrinsic and extrinsic factors affecting the oviposition performance of *S. noctilio* on its host tree *Pinus radiata* D. Don.

Materials, Methods and Results

Laboratory and field studies were conducted at Pittwater, Tas.

Fecundity

The fecundity of newly emerged females was determined by measurement, dissection, and recording the number of eggs in the ovaries. The width of the prothorax was used as the index of size.

The total number of eggs per female in 58 newly emerged insects was described by the equation $y = 28 \cdot 8 (52 \cdot 5)^x$, where y is the total number of eggs per female and x the width of the female's prothorax measured in millimetres (Fig. 1). The computation was based on specimens ranging in size from the smallest (21 mm) to the largest (72 mm) females to emerge from infested trees and billets.

Longevity and Oviposition Rate

Longevity and oviposition performance at constant temperature (26°C) was measured by confining individual females and batches of 10 females in small cages (75 cm³) containing short billets of the host tree. The billets were replaced daily, the phloem removed from the discarded billet and the number and type of oviposition drills recorded. Dead and moribund insects were dissected and any remaining eggs were counted.

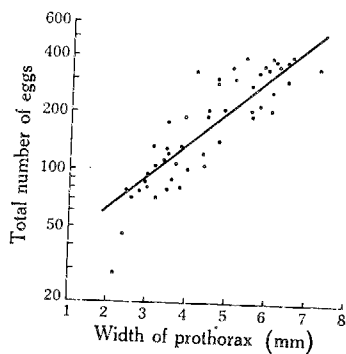


Fig. 1. Egg content in relation to size of *S. noctilio* females. Regression equations: $\log y = 1.46 + 1.72x$; $y = 28.84(52.50)^x$.

Table 1. Longevity and drilling activity of 40 *S. noctilio* females on *P. radiata* billets at 25°C

Days from emergence	Mean prothorax width (mm) ^A	Mean No. of drill groups per female daily	Estimated mean No. of eggs per female daily	Mean No. of unlaidd eggs in dead females
1	4.02	21.8	17.9	10.2
2	5.13	25.4	18.5	5.4
3	6.31	35.7	21.5	0
4	7.50	40.9	25.3	0

^A Of dead females.

Table 2. Drilling of mated and unmated *S. noctilio*
Results are means for six females in each category

Quantity	Mated	Unmated
Thorax width (mm)	4.2	3.8
Longevity (days)	7.8	9.0
Total drill groups	365.3	233.5
Potential No. of eggs	155.7	140.3
Eggs remaining at death	75.3	63.9
Drill groups per day	46.5	23.6
Eggs per day	10.5	6.1

The collective performance and survival of four batches of 10 females held on billets at constant temperature indicated that average longevity was 3 days. Smaller insects died before larger ones and they were not as efficient in disposing of their egg complement. General activity increased with size and the estimated number of eggs deposited per female per day was 18–25 (Table 1). Virgin females were less active than mated females of an equivalent size (Table 2).

Distribution of Eggs in Oviposition Drills

The frequency of eggs in the different types of drills was obtained by dissection of drill sites. The number of eggs per drill, empty drills and the depth of the drills were recorded.

Table 3. Frequency of eggs in single and multiple drills of *S. noctilio*

	Single drills	Double drills	Treble drills	Quadruple drills	Total
Total drills dissected	749	392	172	55	1368
Number with no eggs	718	152	27	2	899
Number with one egg	31	208	56	7	302
Number with two eggs	0	32	70	27	129
Number with three eggs	0	0	18	15	33
Number with four eggs	0	0	1	4	5
Proportion with eggs	0.042	0.610	0.784	0.965	
Mean No. per group with eggs	1.00	1.14	1.92	2.30	
Mean No. eggs per group	0.042	0.684	1.550	2.220	

The presence and frequency of eggs in 1368 drills is summarized in Table 3; the average number of eggs was 0.04, 0.70, 1.60 and 2.30 eggs for single, double, treble and quadruple drills respectively.

Table 4. Distribution of *S. noctilio* drills: coefficients of association for one resistant and three susceptible trees

Asterisk indicates departure from randomness

Tree	Quadrat size(cm ²)	No. of quadrats	Single drills	Multiple drills	Total drills	5% Coefficient limits
Susceptible A	1.31	1596	0.98	1.05	0.98	0.93-1.07
	6.25	399	0.91	1.06	0.90	0.86-1.14
	25.00	100	1.07	0.94	0.88	0.74-1.26
Susceptible B	1.31	936	0.95	0.92	0.86*	0.91-1.09
	6.25	234	0.96	0.84	0.86	0.82-1.18
	25.00	58	0.97	0.77	0.62*	0.66-1.34
Susceptible C	1.31	1008	1.18*	0.94	1.07	0.91-1.09
	6.25	248	1.16	0.92	0.87	0.83-1.17
	25.00	60	0.98	0.109	0.78	0.67-1.33
Susceptible D	1.31	1356	1.01	0.95	0.95	0.93-1.08
	6.25	338	0.92	0.89	0.84	0.85-1.15
	25.00	84	0.99	0.91	0.64*	0.71-1.32
Resistant	1.31	720	1.15	1.00	1.00	0.85-1.15
	6.25	180	1.15	0.82	1.09	0.76-1.24
	25.00	45	2.96*	0.70	4.10	0.53-1.47

Distribution of Oviposition on the Tree Surface

The intensity of oviposition with respect to height and diameter of naturally attacked trees was measured on three 75-cm² areas at regular intervals along their length after telling. The height of the trees ranged from 6 to 25 m. The distribution of oviposition in both killed and resistant trees was found by debarking internodal areas and, after

drying, marking the position of oviposition sites on tracing paper. The papers were subsequently divided into 1.75, 2.5 and 5-cm squares for distribution analysis.

Analysis of the spatial distribution of oviposition types indicated that on successfully attacked trees of uniform bark texture they were distributed at random with a tendency to become uniform. Their distribution on trees which resisted attack tended to become aggregated, i.e. at the time of oviposition the females preferred some regions as opposed to others. The occurrence of multiple drills in resistant trees was generally low and they occurred in discrete, non-continuous areas. The coefficients of association for susceptible trees and a resistant tree are shown in Table 4.

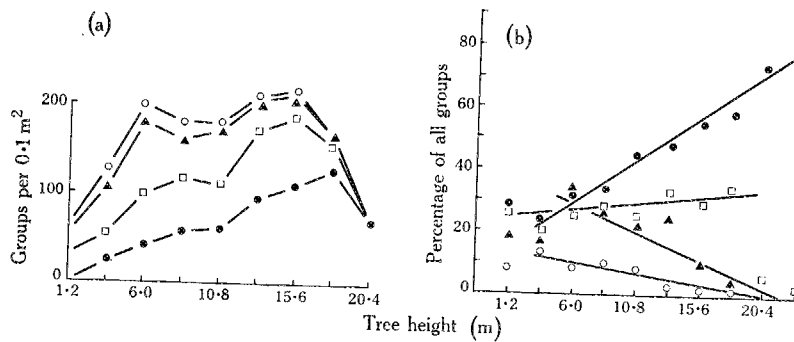


Fig. 2. *S. noctilio* oviposition and height of host trees. (a) Total cumulative density of oviposition groups per unit area at different heights on five *Sirex*-killed codominant trees. (b) The same, but with number of oviposition groups at each height expressed as a percentage of the total number of groups at all heights on all five trees. ○ Quadruple drills. ▲ Trebles. □ Doubles. ● Singles.

The distribution of drill types within large trees indicated trends in which the frequency of singles increased with height, that of trebles and quadruples declined, and that of doubles remained relatively constant. The relative number and proportion of drill types along the length of five mature trees are depicted in Figs 2a and 2b.

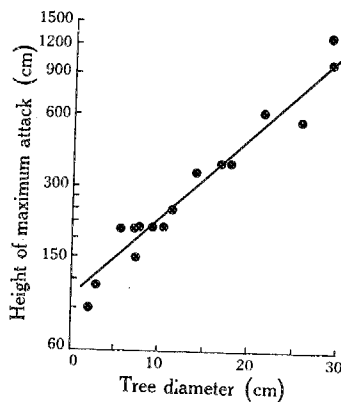


Fig. 3. Height of maximum attack in relation to tree size (measured by diameter over bark 1.25 m above ground level). Regression equations:
 $\log y = 2.008 + 0.034x$;
 $y = 100.20(1.08)^x$.

The relationship between size of tree and height of maximum attack per unit area is shown in Fig. 3. Only trees which contained emergence holes along their complete length were considered. Many trees may not be attacked in their lower regions or they may resist the attack up to various heights.

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Oviposition in Time

The pattern of oviposition after attack was followed by code marking (with coloured pencils) drills made in 0.25- to 0.5-m² areas of bark 0-3 and 6-9 days after attack. These areas were subsequently debarked and the type of each drill recorded for each period of drilling.

A change in the frequency of the different drill types occurred with time after attack (Table 5). Approximately half of the drills in the first 3 days after attack were single ones, whereas from the sixth to ninth days there was an increase in the proportion of treble and quadruple drills.

Table 5. Changes in the proportions of *S. noctilio* drill types with time after first attack

All five trees were 8 cm in diameter at 1.25 m

Tree No.	Drill groups	Proportion of drill groups during:	
		Days 0-3	Days 6-9
1	Single	0.69	0.44
	Double	0.23	0.27
	Treble	0.08	0.19
	Quadruple	0	0.10
2	Single	0.59	0.32
	Double	0.25	0.37
	Treble	0.16	0.24
	Quadruple	0	0.07
3	Single	0.55	0.17
	Double	0.33	0.24
	Treble	0.08	0.55
	Quadruple	0.04	0.04
4	Single	0.26	0.08
	Double	0.39	0.24
	Treble	0.26	0.52
	Quadruple	0.09	0.16
5	Single	0.52	0.31
	Double	0.26	0.25
	Treble	0.22	0.25
	Quadruple	0	0.19
Mean	Single	0.52	0.26
	Double	0.29	0.27
	Treble	0.16	0.35
	Quadruple	0.03	0.12

Effect of Temperature and Humidity on Drilling Activity

A similar procedure was adopted to relate oviposition to temperature and relative humidity. Drills made on billets or trees were marked each hour from 0900 to 1700 h, and temperature and humidity values were obtained from a thermohydrograph in the testing area. The relationship between drilling activity and changes in temperature and humidity is depicted in Fig. 4.

Examination of the individual drilling performance of 15 females of different sizes on single billets held in outdoor cages indicated that maximum rates were associated with temperature and relative humidity of the order of $21 \pm 2^\circ\text{C}$ and 49–52% respectively. When temperatures and humidities from both field and laboratory studies were ranked in order of increasing magnitude, it was found that activity was positively correlated with temperature, increasing to a maximum at 20–22°C and then declining, whereas it was markedly reduced at relative humidities below 40 and above 54%.

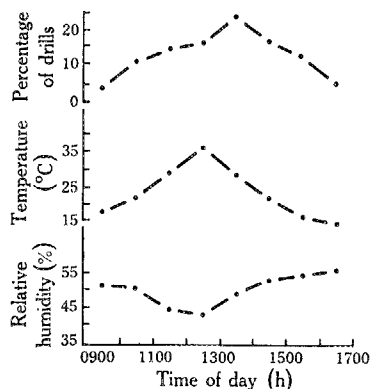


Fig. 4. Drilling activity of *S. noctilio* females with respect to temperature and humidity at different times of day.

Effect of Wood Density on Site Preference

The influence of growth ring width and hence wood density on oviposition was assessed by selecting billets with asymmetric (wide to narrow) growth rings, permitting free attack, and then comparing oviposition on opposite quadrants.

To examine the effect of wood moisture content on oviposition the bark was removed in a single sheet from five billets 7.5 cm in diameter. The wood was cut into equal lengths which were marked and weighed. Pieces were selected at random for storage either at 10°C in a refrigerator or at 100°C in an oven for 1, 4 or 24 h. All bark was held in the refrigerator. Following treatment the pieces were reweighed, the billets reconstituted and the bark stapled into its original location. The inner surface of the bark was dry at attachment and no evidence of a transfer of water from bark to wood was observed when the bark was removed again. All billets were exposed to five females for 72 h, after which oviposition was assessed by removing the bark and counting the drills in both the bark and wood.

There was no significant difference in the average number of drills made in bark and wood with respect to density, i.e. in wood with wide compared with narrow growth rings ($P > 0.05$). A significantly lower number of drills ($P < 0.05$) were made in wood dried for 24 h (estimated moisture content 115%), compared to sections dried for shorter times and with moisture contents of 140–180%.

Phloem Characteristics and Oviposition

The water saturation deficit (W.S.D.) of phloem and the osmotic pressure of phloem sap were the parameters used to evaluate oviposition response to local physiological conditions. The method of Stocker (1929) was employed to measure W.S.D. Fresh billets were exposed to 10 females for 6 h, after which samples of bark were removed with a 1.0-cm cork borer at 2.5-cm intervals on a 20-cm² grid. The bark samples

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were weighed, transferred to distilled water for 16 h, surface-dried, reweighed, then oven-dried for a final dry weight determination. W.S.D. was assessed from the fresh (F) saturated (S) and dry (D) weights of the samples according to the formula $100[(S-D)/(F-D)]$. The bark was then removed from the sample area and oviposition type related to levels of W.S.D.

Van Andel's (1953) differential thermocouple technique was used to determine the osmotic pressure of sap expressed from bark samples. Females were placed on fresh billets in the laboratory, and when a drilling site was vacated the bark containing the drill was removed with a 1-cm borer, transferred to a numbered vial and frozen overnight. The type of drill at each sample site was noted. Sap was expressed from the phloem sample after thawing, collected in a dropper, and a small volume (0.1 ml) placed in a chamber lined with wet filter paper to provide a saturated atmosphere. The chamber was then immersed in a constant-temperature bath at 25°C. A steady thermal e.m.f. was established after 15–20 min and this was recorded on a Phillips microvoltmeter. Osmotic values were obtained by reference to standard curves of e.m.f. generated against either sodium chloride or sucrose solutions of known molarity.

The osmotic pressure was also measured in attacked and unattacked trees at different times and at different heights in the same tree. Dendrometer plates were fitted to a number of test trees to evaluate changes in phloem thickness caused by transpirational load.

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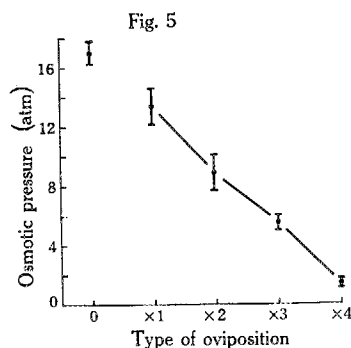
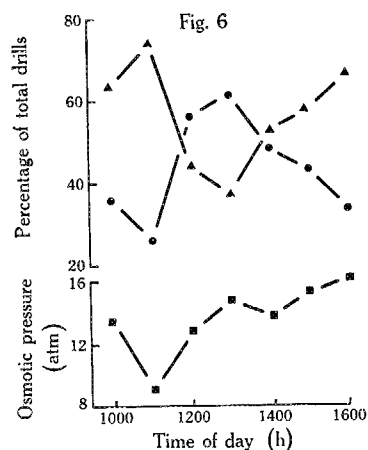


Fig. 5. Type of *S. noctilio* oviposition in relation to the osmotic pressure of phloem sap expressed from the tissues. Mean and standard error for minimum of six samples of each type. 0, dominant unattacked trees. x1, single drills. x2, x3, x4, multiple drills.

Fig. 6. Changes in the type of *S. noctilio* drills with respect to progressive daily changes in osmotic pressure (■). ● Single drills. ▲ Multiple drills.



The type of drill made at a site was related to the osmotic pressure of phloem sap at that site. Single drills were made where pressures were high (> 12 atm) and double, treble and quadruple drills at progressively lower pressure values (Fig. 5). These findings were substantiated by comparing W.S.D. and drill types. Table 6 shows that single drills were made in regions of high W.S.D., i.e. high osmotic potential, with a subsequent shift in type with decreasing W.S.D.

The osmotic pressure of phloem sap changed through the day; more multiple drills were made in the morning, when the average osmotic pressure was low (Fig. 6) and the tissues well hydrated, than in the afternoon.

Dendrometer readings indicated that at 1200 h the phloem thickness was 80% of that at 1000 h, whereas at 1400 and 1600 h it had shrunk to 3 and 1% of the original reading respectively. These changes indicate that phloem hydration undoubtedly affected the general relationships of drill type and average osmotic pressure observed during the morning and under standard laboratory conditions as reported above.

The osmotic pressure of freshly attacked trees was generally lower than that of the nearest unattacked tree of similar class and size. The osmotic pressure of attacked trees declined slowly; however, when the test insects were forced to oviposit on a healthy tree, the average osmotic pressure increased and 8 days later was 5-6 atm higher than that of the unattacked control.

Table 6. Mean water saturation deficit of phloem tissue in the vicinity of *S. noctilio* drills

Billet No.	Mean moisture content (%)	Mean W.S.D.	Mean W.S.D. at drill type:			No. of drills
			Single	Double	Treble	
1	207.0	65.5	65.1	64.9	63.0	59
2	261.0	66.0	67.0	66.2	65.5	21
3	224.0	65.2	65.6	64.2	— ^A	34

^A No treble drills in billet 3.

Table 7. Response of the *S. noctilio* ovipositor to solutions of different molarities

Solutions of water (control) and sodium chloride. Total number of trials 180. Responses: *A*, nil; *B*, flexing; *C*, reciprocation; *D*, pumping; *E*, abdominal movements

Molarity of solution	Number of responses in each category				
	A	B	C	D	E
0	11	101	31	31	6
0.1	4	98	49	32	7
0.2	1	76	61	36	6
0.3	25	66	46	31	12
0.4	46	51	46	20	17
0.5	48	62	25	11	34

Response of the Ovipositor to Direct Stimulation

Isolated abdomens were prepared according to the method of Dethier (1949), to investigate the effect of directly stimulating the ovipositor with osmotically active solutions and solutions of different pH. Female *S. noctilio* were decapitated and their thoraxes embedded in paraffin blocks to make sure the abdomen was free. The tips of the valvae were removed to expose the end of the ovipositor. Abdomens prepared in this way remained responsive for 3-4 days when stored at 10°C between tests. Small quantities of sodium chloride solutions ranging from 0.1M to 0.5M (2-25 atm), were placed in numbered cells of a porcelain spotting plate. Distilled water was used as a control solution and McIlrairie's buffers (pH 3-8) were used to assess response to pH. In a test run the tip of the ovipositor was immersed in distilled water for 10 sec, then for the same time into the test solution and then rewashed. The

response of the ovipositor was assessed subjectively, as no response (*A*), flexing of valvae (*B*), reciprocation of valvae (*C*) and pumping (*D*), and strong abdominal movements (*E*) with no flexing, reciprocation or pumping. Twenty abdomens were employed in the tests and each abdomen was tested nine times over a 3-day period.

The *S. noctilio* ovipositor was insensitive to pH change but solutions of different molarity produced differences in the average response. Flexing and reciprocal movements comparable to those observed in an ovipositing female were elicited predominantly by stimulation of the ovipositor with 0.2M NaCl (9.0 atm) and 0.1M NaCl (4.5 atm) (Table 7).

Discussion

The initial egg content of *S. noctilio* varies from 30 to 450 depending on size; large insects live longer and are more efficient in disposing of their eggs. Newly emerged insects are photopositive and only become ovigerous after a period of flight exercise and mating (author's unpublished information), which would explain the lower performance and activity of unmated females.

The frequency of eggs in the different types of drills indicated that the initial drill is exploratory. Coutts and Dolezal (1969) found that arthrospores and a mucosecretion were placed in this type of drill and in the empty drill(s) of the multiple types. The empty drill was invariably the last drill made at a site. Oviposition sites are randomly distributed upon the tree with a tendency to become uniform. However, the distribution of drills in some resistant trees tended to become aggregated, which indicates that some areas of the trees' surface were more acceptable than others. The frequency of drill types also varied with height and in time after attack. The intensity of attack along the length of a tree varied, but the height of maximum attack was found to be generally related to size of the tree, this relationship being related to the physiological status of the tree at the onset of attack. The respiratory activity of the phloem tissue in normal, unattacked trees is not constant but shows a maximum rate at a height which is proportional to tree size (author's unpublished observations). Above and below this region the rate declines. Under conditions of stress the overall respiration of the tree increases, hence an exhaustion of respiratory substrate with a subsequent decline in osmotic pressure occurs first in the region of maximum rate and it is this region which is first attacked under field conditions (author's unpublished observations.)

The oviposition behaviour of the woodwasp represents an economy in the deposition of eggs which is related to the condition of the host tree. Single drills are made in those sites where osmotic pressure and diffusion pressure deficit measurements indicate 'normal' physiological conditions; hence, more single drills are made in the initial stages of attack. The injection of mucus and arthrospores into the tree at this time reinforces the initial stress condition so that the vigour of the tree progressively declines. Oviposition behaviour is modified by these changes in the host tree and greater numbers of eggs are deposited. Chararas (1959) reported that a gradient in osmotic pressure existed along the length of *P. maritima* trees with higher values recorded at the upper levels. The same condition prevails in *P. radiata* (unpublished information); however, it was found that osmotic pressure varied between adjacent samples so that internodal areas consisted of a mosaic of osmotic pressure values. This distribution was also reflected in the W.S.D. values.

S. noctilio drills

No. of drills
59
21
34

Coutts (1965) reported that a high proportion of single drills occurred when the general moisture content (wood and bark) was excessively high (>180%) or low

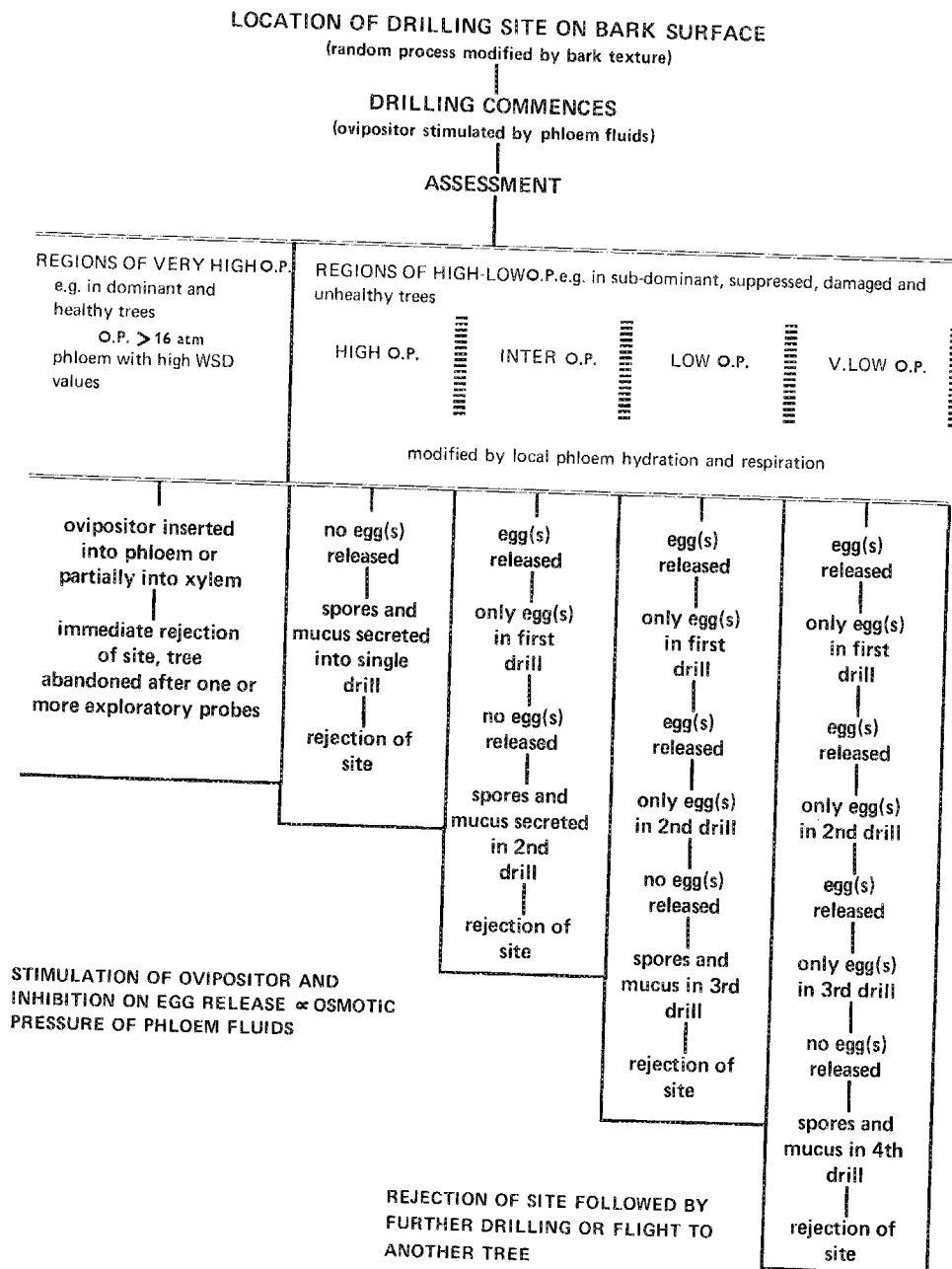


Fig. 7. Possible mechanism by which *S. noctilio* regulates egg deposition with respect to the physiological status of the host tree, *P. radiata*. O.P., osmotic pressure.

(<60%). In the present investigation it was found that the frequency and type of oviposition drills were not affected by wood density (growth rings) or wood moisture

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content in the 140–180% range. These results further emphasize the role of the phloem in determining oviposition activity.

Direct stimulation of the ovipositor with osmotically active solutions resulted in a graded response. Solutions of 4.5 and 9.0 atm, osmotic pressures which in the phloem sap facilitated multiple drills, promoted the highest frequency of movements consistent with normal drilling.

The relative frequency of double drills is generally unrelated to height (cf. Fig. 2*b*) and time (Table 5) in any given tree, and it was observed that the tissues in which double drills were made were often lacking in extractable sap. No such difficulty was experienced with the tissues containing more or fewer drills. It appears that tracts of tissue do exist, even in healthy trees, in which moisture content is at a critical minimum or alternatively the tissues are not actively metabolizing and growing.

The regulation of egg deposition with respect to the osmotic status of the phloem sap is presumably mediated through the central nervous system. The tip of the ovipositor carries sensory pits, and dissection of moribund insects has shown that the presence of an egg in the vaginal vestibule effectively occludes the openings of the intersegmental sacs, which harbour the arthrospores, and the duct from the mucus reservoir. Hence perception of a stimulus from the phloem at the onset of drilling results in either no egg being released from the oviduct (single drill with arthrospores and mucus) or the release of eggs at a rate related to the strength of the stimulus; thus double, treble and higher but less frequent drill types are made. This possible mechanism is represented in Fig. 7.

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