THE INFRABUCCAL CHAMBER OF *CAMPONOTUS MODOC* (HYMENOPTERA: FORMICIDAE): INGESTION, DIGESTION, AND SURVEY OF BACTERIA

LAUREL D. HANSEN, WALTER J. SPANGENBERG, AND MELISSA M. GAVER
Biology Department, Spokane Falls Community College, 3410 W. Fort Wright Drive Spokane, WA 99224-5288 USA

Abstract - Carpenter ants feed on both liquid and solid food. Baiting for carpenter ants has proceeded using both of these media to introduce toxicants into the colony for control. Ingestion of food particles larger than 100 microns is effectively curtailed by the specializations of the epipharynx, hypopharynx, and various maxillary and labial structures. These filtration mechanisms function as food enters or exits the infrabuccal chamber (pocket), an oral storage area. Solid food materials are taken into the infrabuccal chamber of *Camponotus modoc* Wheeler workers where secretions from head and thoracic glands aid in digestion. A liquid diet can then pass through the filtering mechanism as it exits the infrabuccal chamber into the pharynx. It was hypothesized that the infrabuccal chamber contained bacteria that may also be a factor in digestion and ultimately in the dissemination of toxicants in baits. Using sterile technique, infrabuccal chambers were removed via microdissection from the heads of ants. The contents were cultured on Muller Hinton, R 2-A, and chitin agar. Plates were incubated at room temperature under aerobic, microaerophilic, and anaerobic conditions. The mixed microbial flora were predominately Gram positive rods in the genus *Bacillus*. Gram positive cocci, Gram negative rods, and yeasts were also found.
Key words - Carpenter ants, feeding, bacteria, digestive glands, baits

INTRODUCTION

The generalized mouth parts of insects include the labrum, mandibles, maxillae, and labium. In the ants as in most Hymenoptera, the maxillae and labium form a maxillo-labial complex forming a channel along which food is passed to the mouth. Mouth parts of ants have been extensively studied by Gotwald (1969). An important phase of food ingestion occurs in a preoral area produced by the mouth parts and the hypopharynx. The hypopharynx, together with the epipharynx forms a preoral food chamber which opens into a second chamber, the infrabuccal chamber or pocket. This structure is an effective filtering device which prevents particles larger than 100 microns from entering the alimentary canal in *Camponotus pennsylvanicus* DeGeer (Eisner and Happ, 1962). This sizable chamber collects solid food particles and other materials and compacts them. The literature on the fate of this formed pellet is sparse. It is reported to be waste in *Acromyrmex* but is fed to the larvae of arboreal *Pseudomyrmex* ants. The fact that this pellet has not been observed in culture dishes with workers of *C. modoc* Wheeler and that all workers during the foraging season contain a mass within the infrabuccal chamber precipitated this study of the feeding structures, glands associated with the infrabuccal chamber, and the possibility of symbiotic bacteria within this chamber.

Functions of many glands in the head of the ant have not been determined. A morphological study of the digestive system with associated glands was made by Forbes (1938) and Forbes and McFarlane (1961) in *C. pennsylvanicus*. They report that the maxillary glands, labial glands, and the post pharyngeal glands serve a digestive function. The maxillary and labial glands may also have a role in trophallaxis. Ayre (1963) found the labial gland serves primarily as a source of the digestive enzyme, amylase, but questions the digestive activity of secretions from the postpharyngeal glands in *C. herculeanus* (L.). He noted the absence of protease in these digestive glands.

Although intracellular symbiotic bacteria have been discovered in the ovarioles and midgut epithelium of *C. herculeanus* and *C. ligniperda* Latr. (Buchner, 1965), no investigations of symbiotic bacteria in the infrabuccal chamber have been made.

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

The acceptance of baits and efficacy of baiting programs have had limited success in carpenter ant control (Hansen, 1996). These studies were initiated to further the understanding of food ingestion by carpenter ants.

MATERIALS AND METHODS

Carpenter ant colonies of *C. modoc* were collected in north Idaho in1996 and 1997; a colony of *C. herculeanus* was collected on the Kenai Peninsula, Alaska in 1996. Colonies were maintained in the laboratory in large culture containers with honey and protein. Medium and large workers were selected for dissection of mouth parts, head and thoracic glands, and infrabuccal chambers.

Morphology. Dissections were made of over 25 workers of *C. modoc.* The mouth parts and glands in the head and thoracic glands were studied in freshly killed or living material in a 1.0% solution of sodium chloride or 70% ethanol. Fat cells and muscle tissues were removed as well as could be managed to observe glands and ducts. Structures of the head and thorax were photographed and illustrated..

Removal of infrabuccal chambers. To remove surface bacteria, twenty *C. modoc* workers were immersed one minute in 0.5% sodium hypochlorite solution. Within a laminar flow hood, one side of the head was excised and infrabuccal chambers and contents were removed with sterile forceps (Fig. 1). The procedure was initiated 19 June 1996 (Set A) and replicated 25 June 1996 (Set B), 19 July 1997 (Set C), and 10 September 1997 (Set D). The procedure was repeated in August 1996 (Set E) with *C. herculeanus.*

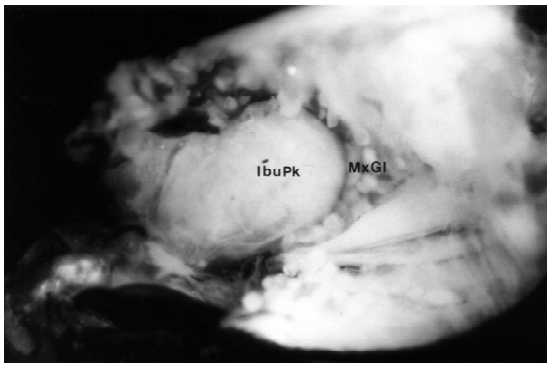

Figure 1. Parasagittal section through the head capsule of a *Camponotus modoc* worker. IbuPk, infrabuccal pocket; MxGl, maxillary gland.

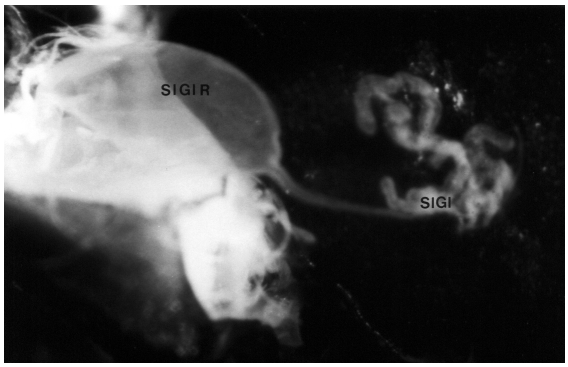

Figure 7. Parasagittal section through the prothorax of a *Camponotus modoc* worker showing salivary gland reservoir (SlGlR) and gland (SlGl).

Determining number and isolation of bacteria. Using sterile technique, each infrabuccal chamber and its contents, in a sample of ten chambers, were weighed to the nearest microgram. Each infrabuccal chamber and its contents were added to 190 ml of sterile water and vortexed vigorously. Serial dilutions were made to 10^{-6}. A 100 ml sample of each dilution was plated on nutrient agar and 0.8% chitin agar and incubated at 30° C for 48 hrs under aerobic conditions.

A single infrabuccal chamber was added to 5 ml of sterile water on the surface of either a R 2-A, glucose-yeast extract-chitin agar (GYCA), Muller Hinton agar (MHA), or 0.8% chitin agar (CA) plate. After mixing, the contents were streaked for colony isolation. Plates were incubated at room temperature under aerobic, anaerobic, and microaerophilic conditions for 48 hrs. Three infrabuccal chambers including their contents were added to 3 ml of glucose-yeast extract-chitin broth (GYCB), or Muller Hinton broth (MHB) and incubated at room temperature under aerobic, anaerobic, and microaerophilic conditions for 48 hrs. A sample of each culture was then streaked for colony isolation on R 2-A, GYCA, MHA, or CA plates and incubated at room temperature under aerobic, anaerobic, and microaerophilic conditions for 48 hrs.

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

All plates were surveyed with both the unaided eye and under a dissecting microscope to determine unique colony characteristics. A sample of each colony type was subcultured on appropriate media at room temperature. Culturing under anaerobic and microaerophilic conditions was terminated at this time due to lack of growth. Bacteria from individual colonies were grouped by the properties of Gram staining, spore staining, cell morphology, and cell size. Each isolate was tested for 38 different biochemical activities using the Becton Dickinson Crystal Identification Systems Enteric Nonfermenter ID Kit and Rapid Stool Enteric IC Kit. From the 66 isolates for which there was a biochemical profile, 10 isolates were selected and sent to Microcheck, Inc., a commercial microbial analytical laboratory. Microcheck, Inc. made identifications with a 70-99% accuracy by analyzing the fatty acids content of each isolate with gas chromatography.

Optimum growth temperature. From Sets A and B, 34 organisms were selected from the 47 isolates and cultured in R 2-A broth at 35°C for 24 hrs. A 150 ml sample was then added to 985 ml of sterile R 2-A broth. A BIOLOG spectrophotometer was used to determine optimum growth by measuring the lowest percent transmission at 590 nm. Cultures were measured following 5, 10, 18, and 46 hrs growth.

RESULTS

Morphology. Food is effectively filtered in two areas before entering the pharynx. First through the mouth parts before entering the infrabuccal chamber and secondly as it leaves the infrabuccal chamber and enters the pharynx. In the preoral area filtering occurs as food is passed over the maxillae and labium. On the maxillae numerous thin setae plus the galeal, maxillary, and lacinial combs filter food as it enters this preoral cavity (Fig. 2). Food then passes over the glossa of the labium into the infrabuccal chamber filtered by additional setae (Fig. 3). The opening to the salivary duct serves as a marker between the hypopharyngeal region and the prementum of the labium. The ridges of the glossa close against the buccal plate, which forms the dorsal side of the chamber (Fig. 4). The buccal plate is thick with hair-like projections on its walls which point toward the entrance to the chamber.

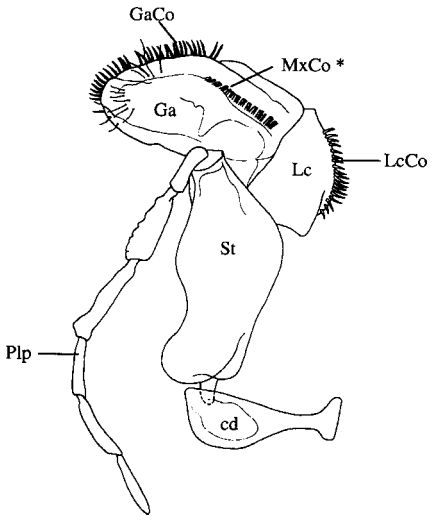

Figure 2. Maxilla of a *Camponotus modoc* major worker, external surface. Maxillary comb (MxCo) on internal surface has been drawn as though the galea were transparent. Cd, cardo; Ga, galea; GaCo, galeal comb; Lc, lacinia; LcCo, lacinial comb; Plp, palp; St, stipes.

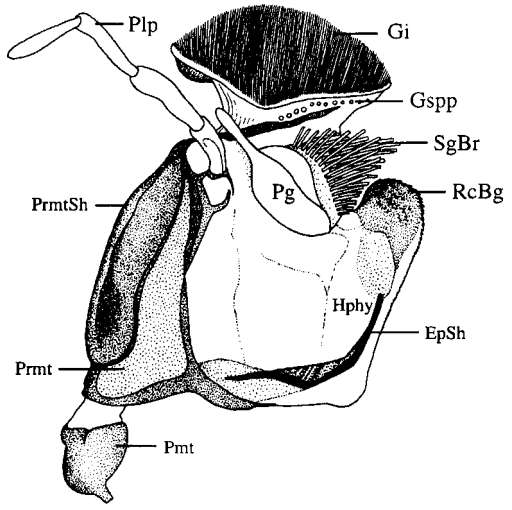

Figure 3. Labium of a *Camponotus modoc* major worker, lateral view. EpSh, epimental shield; Gl, glossa; Gspp, Gustatory papillae; Hphy, hypopharynx; Pg, paraglossa; Plp, Palp; Pmt, postmentum; Prmt, prementum; PrmtSh, premental shield; RcBg, Racquettes of Bugnion; SgBr, subglossal brush.

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

As food is pumped out of the chamber into the pharynx, the buccal plate meets with the posterior surface of the epipharynx which contains ridges and hairs projecting toward the infrabuccal chamber (Fig. 5). The ”buccal tube” of Forbes (1938) and Forbes and McFarlane (196l) is not really a tube but a layer of tissue which is an extension from the posterior surface of the pharynx extending into the dorsal surface of the infrabuccal chamber. The pharynx is flattened dorsoventrally with the anterior area sclerotized to form a thin plate. This plate compresses against the buccal plate which bears many rows of hairs projecting toward the infrabuccal chamber. This provides the final filtering mechanism as liquid food is ingested into the system.

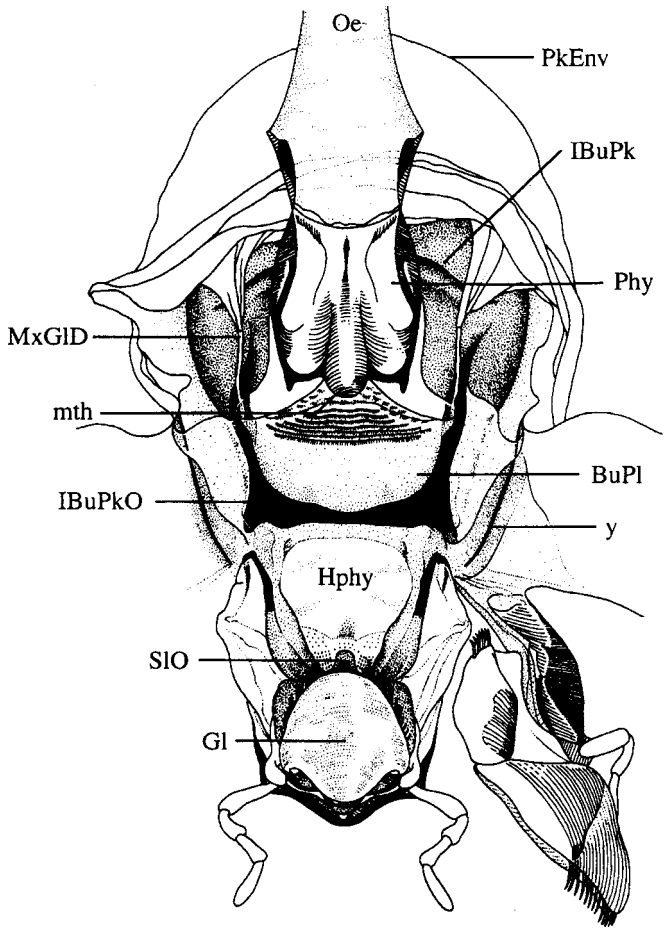

Figure 4. Anterior view of the cibarium of a *Camponotus modoc* major worker with epipharynx removed showing entrance into the infrabuccal chamber. BuPl, buccal plate; Gl, glossa; Hphy, hypopharynx; IBuPk, infrabuccal pocket; IBuPkO, infrabuccal pocket orifice; Oe, esophagus; MxGlD, maxillary gland duct; mth, mouth; Phy, pharynx; PkEnv, pocket envelope; SlO, salivary orifice; y, suspensoria.

A number of glands associated with the mouth parts are present in the head (Fig. 6). The postpharyngeal glands located in the head above the pharynx, are composed of two large groups of finger-like structures. They extend anteriorly over the pharynx and posteriorly into the head. Openings for these glands are located at the junction of the pharynx and the esophagus. Propharnyngeal glands are located on the anterior surface of the pharynx and ducts open into the pharynx. The maxillary glands lie on either side of the pharynx near the infrabuccal chamber into which the ducts from these glands open. The labial or salivary glands, located in the dorsolateral regions of the thorax, produce secretions which are stored in reservoirs in the prothorax and empty through ducts

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

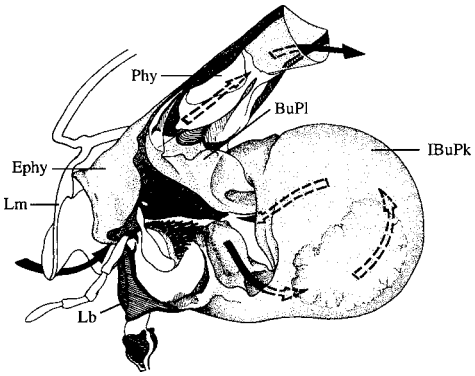

Figure 5. Lateral view of the feeding apparatus of a *Camponotus modoc* major worker showing route of the food through the cibarium, infrabuccal chamber, and pharynx. BuPl, buccal plate; Ephy, epipharynx; IbuPk, infrabuccal pocket; Lb, labium; Lm, labrum; Phy, pharynx.

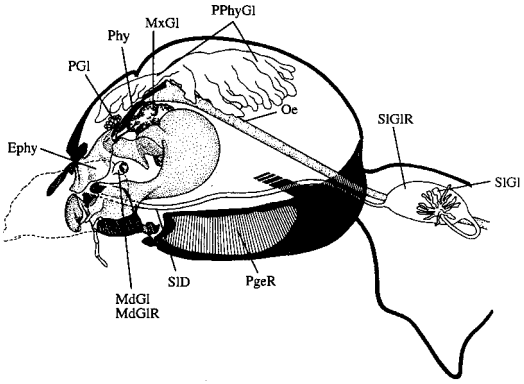

Figure 6. Lateral view of the glands of the head appendages and other anterior glands in a *Camponotus modoc* major worker. Ephy, epipharynx; MdGl, mandibular gland; MdGlR, mandibular gland reservoir; MxGl, maxillary gland; Oe, esophagus; PgeR, postgenal ridge; PGl, propharyngeal gland; PphyGl, postpharyngeal gland; Phy, pharynx; SlD, salivary duct; SlGl, salivary gland; SlGlR, salivary gland reservoir.

which lie near the esophagus. The ducts extend into the head and unite as one duct which opens between the hypopharynx and the labium. Thus both secretions of the labial and maxillary glands will contact food as it enters the infrabuccal chamber. Secretions of the propharyngeal and postpharyngeal glands also may be regurgitated into this chamber.

Number and identification of bacteria. The weight of each of the 10 chambers ranged from 0.4 to 0.1 mg with an average weight of 0.19 mg. Using the 10^{-6} dilution factor, the average of 369 colonies on nutrient agar plates, and 297 colonies on chitin agar plates, an estimate was determined of 16.7×10^4 bacteria per mg of contents in the infrabuccal chamber.

The number of different colony types varied from 15 to 26 among the *C. modoc* samples, with higher numbers occurring in the peak of foraging activity (Table 1). The total number of isolates investigated in *C. modoc* was 82. During the course of investigation some isolates were discarded because they

Table 1. Organisms isolated from the infrabuccal chambers of *Camponotus modoc* and *C. herculeanus*.

	Camponotus modoc				*C. herculeanus*
	Set A	Set B	Set C	Set D	Set E
No. of organisms	26	21	20	15	22
Bacteria					
Gram positive rods	18	16	8	4	5
Gram negative rods	4	2	4	7	3
Undetermined rods	4	0	0	0	2
Spore forming	22	12	3	0	5
Gram positive cocci	0	3	8	3	1
Yeast	0	0	0	1	13

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

Table 2. Bacteria selected from cultures of infrabuccal chambers of *Camponotus modoc* workers from Sets A and B and identified by Microcheck.

Set	Isolate	Identification	Similarity Index*	Probability
A	Q-2	*Bacillus licheniformis*	0.619	
	S-1	*B. sphaericus*	0.735	
B	D	*B. thuringiensis*	0.417	
	G	*B. thuringiensis*	0.510	
	I-1	*B. licheniformis*	0.581	
	K	*B. pumilus*	0.408	
		B. licheniformis	0.407	
	A	*Serratia liquefaciens*		98%
	C	*Pantoea agglumerans*		99
	N	*Leclercia adecarboxyla*		89
	Q	*Klebsiella pneumoniae ozaenae*	0.801	
*Similarity Index:		0.999 to 0.555 = excellent match		
		0.301 to 0.499 = good match		
		0.100 to 0.300 = may not be good comparison		

were replicates and some isolates failed to survive subculturing. In the final isolate pool which consisted of 66 cultures, 37 were Gram positive rods and formed spores. It is hypothesized that all of these organisms will be found to be in the genus *Bacillus.* Of the 10 cultures identified by Microcheck, six were placed in the genus *Bacillus* (Table 2). In removing infrabuccal chambers for the September samples, approximately one-half of the ants had little or no material in the infrabuccal chamber and were not used in the procedure.

When comparing the pattern of positive biochemical tests of the bacteria that were identified by Microcheck with the pattern of positive biochemical activities for the unidentified isolates, there is a large number of bacteria that were not identified. Of the original 104 isolates cultured in this investigation only 19 have been identified with reasonable accuracy (Table 3). The most common type of microorganism found in *C. herculeanus* was yeast. Thirteen different yeast colonies and nine bacterial colonies were identified. The bacteria and yeast were not identified to species. It is interesting to note that of the 66 isolates from *C. modoc*, only one was a yeast colony.

Bacterial growth. Three of the five sets included a test to determine if there were bacteria within the infrabuccal chamber that could utilize chitin as their sole source of nutrients. Of the 56 isolates within these three sets, 13 exhibited the capacity to utilize chitin (Table 4). Using the 34 isolates from sets A and B, it was determined that there was a range of optimum temperatures at which the bacteria grew in the infrabuccal chamber. The optimum temperature for the majority of the isolates was 33° C (Table 5).

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

Table 3. Identification of bacterial isolates from infrabuccal chambers of *Camponotus modoc* workers by comparison of the number of matching biochemical tests (Becton Dickinson Crystal Identification Systems Enteric Nonfermenter ID Kit and Rapid Stool Enteric IC Kit) to Microcheck identifications.

Set	Isolate	Microcheck Identification	Number of Matching tests	Biochemical match
A	Q-2	*Bacillus licheniformis*	21/21	100%
	Q-3		19/21	91
	Q-4		19/21	91
	R-1		19/21	91
	S-1	*B. sphaericus*	21/21	100
B	D	*B. thuringiensis*	18/18	100
	H		15/18	83
	G	*B. thuringiensis*	11/11	100
	F		9/11	82
	I-2	*B. licheniformis*	14/14	100
	I-1		14/14	100
	X		12/14	86
	K	*B. pumilus*	14/14	100
	L		13/14	93
	A	*Serratia liquefacians*	23/23	100
	C	*Pantoea agglumerans*	16/16	100
	B		15/16	94
	N	*Leclercia adecarboxyla*	19/19	100
	O	*Klebsiella pneumoniae ozaenae*	9/9	100

Table 4. Bacteria isolated from infrabuccal chambers of *Camponotus modoc* workers from Sets B, C, and D and cultured on 8% chitin agar.

Bacterial Isolates	Set B	Set C	Set D
Total number of organisms	21	20	15
Number of organisms with growth on chitin agar	8	2	3
Gram positive, Spore forming rods	4*	1	0
Gram positive rods	1	1	0
Gram negative rods	0	0	2**
Gram positive cocci	3	0	1

*2 isolates were identified by Microcheck as *B. thuringiensis*

**1 isolate was identified by Microcheck as *Serratia liquefaciens*

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

Table 5. Number of cultures from infrabuccal chambers of *Camponotus modoc* (Sets A and B) that achieved maximum growth at temperature ranges 23-42 ºC for 5-26 hrs. (Determination made by the lowest percent transmission on a BIOLOG spectrophotometer.)

Incubation	Culture times			
Temperature	5 hr	10 hr	18 hr	26 hr
23°C	6 cultures	4 cultures	9 cultures	8 cultures
30	8	9	6	8
33	9	19	10	14
37	11	2	9	4
42	0	0	0	0

DISCUSSION

Ants ingest both liquid and solid foods in their foraging activities. The assumption is made that the liquid food passes directly to the pharynx and then through the esophagus for storage in the crop. Solid food, however, is prevented from entering the pharynx by the unique features of the mouth parts and by the ultra-filtering mechanisms as food is passed from the infrabuccal chamber to the pharynx. With the physical reduction in size of food particles and the addition of digestive enzymes, digestion must be initiated in this pre-pharyngeal area of the ant. The large size of the infrabuccal chamber and the retention of the contents suggest additional activity such as that found in symbiotic organisms. This survey of bacteria in the role of ingestion and digestion needs further investigation, particularly as control procedures for carpenter ants are including the introduction of solid baits.

The bacteria identified in this study belong to two families, Bacilliacae and Enterobacteriacae. Both of these families of bacteria are widely distributed in soil and water as well as on plant surfaces. The members of the genus *Bacillus* are diverse in their physiological abilities. There are thirty-five species in the genus *Bacillus*, many common to the soils of Eastern Washington. Two very common species *B. cereus* and *B. subtilis* were not identified in the infrabuccal chambers. The genus *Pseudomonas* also is ubiquitous in the environment. If carpenter ants obtain bacteria in their infrabuccal chamber by ingesting soil along with their prey, these species would be part of the microbial flora.

Although anaerobic incubation of the infrabuccal chamber contents was discontinued early in this investigation, it is hypothesized that anaerobic microorganisms reside inside the chamber. All the bacteria identified by Microcheck were facultative anaerobes which would be consistent with large numbers of bacteria metabolizing within a small space with limited oxygen. Also, the majority of Gram positive rods isolated were spore-formers indicating that they belong to the genus *Bacillus*. Of the 35 species in this genus, 34 are facultative anaerobes. The Gram negative rods identified were in the family Enterobacteriacae, a family of facultative anaerobes.

To find bacteria within the infrabuccal chamber that could utilize chitin as a sole source of nutrient was not surprising. Investigations into the nutritional preferences of the bacteria that could utilize chitin were not done. However, it is hypothesized that there are no exclusive chitin metabolizers residing in the chamber because of the rich environment that exists there from body tissues and fluids of prey.

This cursory investigation of the bacteria in the infrabuccal chamber generates numerous questions such as: What is the length of time that food is retained in the infrabuccal chamber? Is there a mechanism with which carpenter ants control the kinds of bacteria that reside in the infrabuccal chamber? Do the bacteria contribute any essential nutrients; i.e., vitamins, fatty acids, amino acids? Is there a different population of bacteria in the infrabuccal chamber of different species of carpenter ants? Is there a seasonal change in the kinds of bacteria in the infrabuccal chamber? Is there a change in the kinds of bacteria in the infrabuccal chamber in ants of the same species from different geographical locations? Can

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.

polymerase chain reaction (PCR) be used as a genetic probe for investigating the kinds and distribution of bacteria in the infrabuccal chamber? Can the bacterial population in the infrabuccal chamber be altered to control carpenter ant populations?

REFERENCES CITED

Ayre, G. L. 1963. Feeding behaviour and digestion in *Camponotus herculeanus* (L.) (Hymenoptera: Formicidae). Entomol. Exp. et Appl. 6: 165-170.

Buchner, P. 1965. Endosymbiosis of Animals with Plant Microorganisms. Interscience Publishers, John Wiley, New York. 909pp.

Eisner, T. and G. J. Happ. 1962. The infrabuccal pocket of a formicine ant: a social filtration device. Psyche 69: 107-116.

Forbes, J. 1938. Anatomy and histology of the worker of *Camponotus herculeanus pennsylvanicus* DeGeer (Formicidae, Hymenoptera). Ann. Entomol. Soc. Am. 31: 181-195.

Forbes, J. and A. M. McFarlane. 1961. The comparative anatomy of digestive glands in the female castes and the male *Camponotus pennsylvanicus* DeGeer (Formicidae, Hymenoptera). J. New York Entomol. Soc. 69: 92-103.

Gotwald, W. J. 1969. Comparative morphological studies of the ants, with particular reference to the mouth parts (Hymenoptera: Formicidae). Cornell Univ. Agric. Exp. Sta.: Ithaca, NY. Memoir 408. 150p.

Hansen, L. D. 1996. Managing carpenter ant populations in urban environments. In Wildey, K. B., ed, Proc. 2nd Inter. Conf. Insect Pests Urban Environ. Exeter, Great Britain: BPC Wheatons, pp. 587-595.

Proceedings of the 3rd International Conference on Urban Pests.
Wm H. Robinson, F. Rettich and G.W. Rambo (editors). 1999.