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Cuticular metal hardening of mouthparts and claws of some forest insects of British Columbia.

A. R. FONTAINE, N. OLSEN, R. A. RING and C. L. SINGLA

DEPARTMENT OF BIOLOGY, UNIVERSITY OF VICTORIA
VICTORIA, B.C., V8W 2Y2

ABSTRACT

The presence of metals in mouthparts and claws of some forest insects associated with British Columbia conifers, particularly cone and seed pests, were detected and mapped by energy dispersive X-ray microanalysis. Zinc was concentrated in the mandibular cutting edges and claw tips of larval lepidopterans (but not in adult mouthparts), in the mandibles and claws of larval and adult coleopterans and in the mandibles of the hymenopteran, *Megastigmus spermatotrophus*. Calcium was the predominant metal in the mouth hooks of dipteran larvae, but minor peaks of zinc or manganese were present additionally in two species. Manganese occurred in the stylets of the hemipteran, *Leptoglossus occidentalis*, in the mandibles and claws of one coleopteran species, and with zinc in the mandibles of a clerid predator. The function of metal concentrations in specific areas of these structures is probably related to hardening of cuticular regions in some instances and to some other biomechanical aspect of cuticular strengthening in other cases.

INTRODUCTION

Forest insect pests, especially those that feed on developing cones and seeds or damage potential seed-bearing branches, have a major economic impact on coniferous forest productivity and regeneration in British Columbia (Ruth, 1980; Wood and Van Sickle, 1987). It is of interest to forest entomologists, therefore, to know that mouthparts and claws of some of these herbivorous pests appear to be hardened by cuticular metal deposits that may affect abrasive wear or confer strength biomechanically. We have surveyed some B.C. forest insects, emphasizing seed and cone pests, for evidence of cuticular metal hardening using energy dispersive X-ray microanalysis (EDXa) to detect metals, and X-ray mapping to show their morphological distribution. The information presented here has implications in the ecology of forest insect pests and is a base for studies on the interrelationship between hardening of insect mouthparts, particularly during development, and insect feeding strategies.

Use of metals, such as iron, zinc, copper, manganese or silicon, to harden mouthparts and other structures as an adaptation against excessive wear is widespread among both aquatic and terrestrial invertebrates (Simkiss and Wilbur, 1989). This adaptation also occurs in insects, though its extent is not particularly well known. Concentration of zinc in the mandibles of two locust species, the original accounts of cuticular metal hardening in insects, was related to resistance to wear in relation to feeding on tough plant material (Hillerton and Vincent, 1982; Hillerton, Reynolds and Vincent, 1982). Hillerton and Vincent (1982) also used EDXa and X-ray mapping to demonstrate the specific location of zinc or manganese along the cutting edge of chewing structures in 36 herbivorous species from 5 orders; five omnivores from 2 orders did not have metals in their mouthparts. Subsequently Hillerton, Robertson and Vincent (1984) demonstrated zinc or manganese in the mandibles of 54 (out of 57) species of stored-product beetles, thus emphasizing the major role of these metals in increasing the hardness of chewing structures. Co-occurrence of metals, i.e., two metals occurring in the same structure, has been reported in some species, but its significance is not well understood. For example, ion microprobe techniques have demonstrated concentrations of both zinc (about 4%) and manganese (about 0.4%) in the mandibular teeth of ants (Lefevre *et al.*, 1987; Schofield *et al.*, 1988).

Calcium, the only other metal commonly found in insect cuticle, is prominent in Diptera, notably in muscid flies where its presence has been related functionally to stabilization of the puparial cuticle as an alternative to sclerotization, not as a hardening mechanism to resist abrasion (Grodowitz and Broce, 1983; Roseland *et al*, 1985).

MATERIALS AND METHODS

Specimens of insect larvae and adults (Table 1) were obtained from culture stocks and collections of the Pacific Forestry Centre, Victoria, B.C., through the assistance of Mr. D. S. Ruth. Usually they were received preserved in 70% ethanol after previous fixation, but some live specimens were fixed by us in 2.5% glutaraldehyde in phosphate buffer, pH 7.4.

The appropriate mouthparts (mandibles, stylets, or mouth hooks) and claws were removed, dehydrated in a graded ethanol series and air dried; alternatively they were removed after dehydration and critical point drying. Dried structures were mounted on carbon boats using carbon paste, then lightly sputter coated with gold. Although artifactual gold peaks were thus introduced, gold-coating reduced the extreme charging problems encountered in carbon-mounted specimens. EDXa was performed with a Tracor Northern 5500 EDXa system mounted on a JEOL 1200EX scanning transmission electron microscope operated in the scanning electron microscope (SEM) mode. X-ray spectra were typically acquired from specimens tilted to 30° for 100 seconds over the energy range 0-20 keV, at an accelerating voltage of 40 kV, beam current 15 mA. Digital video images of the specimens and corresponding X-ray maps, with appropriate controls, were acquired and processed using Tracor Northern software. Some SEM morphology was carried out

Table 1
Taxonomic list of the forest insects surveyed, including stage of development and collection data.

Species	Common name	stage of life cycle	Collection data
LEPIDOPTERA			
Barbara colfaxiana (Kearfott)	Douglas fir cone moth	eggs instars 1-4 adult females	Keremeos B.C. 18/5/87 cone collection 28/6/84
Cydia strobilella (L.)	Spruce seed moth	instar 1 instar 4	Tappen, B.C. 26/5/87 White spruce cones, Smithers, B.C. 6/8/68
Dioryctria abietivorella (Grote)	Fir coneworm	larva	Mesachie Lake, B.C. 18/8/80
Dioryctria reniculelloides Muutura and Munroe	Spruce coneworm	larva	Oliver, B.C. 26/5/87
Holocera immaculella McDermott	Douglas fir fall coneworm	larva	Hedley, B.C. 2/6/87
DIPTERA			
Contarinia oregonensis Foote	Douglas fir cone gall midge	larva	Mesachie Lake, B.C. 15/8/86
Delia anthracina (Czerny)	Spiral spruce cone maggot	instars 1-4 eggs	White spruce cones, Prince George, B.C. 11/6/87 Tappen, B.C. 26/5/87
Earomyia abietum McAlpine	Fir cone maggot	larva	Grand fir cones, Ladysmith, B.C. 6/8/68
Earomyia barbara McAlpine	Fir cone maggot	larva	Douglas fir cones, Ladysmith, B.C. 28/8/72
HEMIPTERA			
Leptoglossus occidentalis Heidemann	Seed bug	instars 1, 2 adult	lab rearings 22/5/87 lab rearings 8/5/87
HYMENOPTERA			
Megastigmus spermatotrophus Wachtl	Douglas fir seed chalcid	larva	Douglas fir cone seed, Courtney, B.C. 19/9/71
COLEOPTERA			
Dendroctonus ponderosae Hopkins	Mountain pine beetle	instars 3, 4; adults	from Lodgepole pine held in cold storage. 15/7/87
Enoclerus schaefferi Barr	Checkered beetle, predator upon *B. colfaxiana*	larva	cone collection, Keremeos, B.C. 14/5/87
Neacanthocinus obliquus Le Conte	Long-horned wood borer	adult	lab rearings 15/7/87

Table 2
Metals in the feeding structures and claws of forest insects demonstrated by EDXa.

Species	Stage	Structure	Metals
LEPIDOPTERA			
Barbara colfaxiana	eggs		none
	instars 1-4	mandibles	Zn
		claws	Zn
	adult	mouthparts	none
Cydia strobilella	instar 1	mandibles	Zn
		claws	Zn
	instar 2	mandibles	Zn
		claws	Zn
Dioryctria abietivorella	larva	mandibles	Zn
		claws	Zn
Dioryctria reniculelloides	larva	mandibles	Zn
		claws	Zn
Holocera immaculella	larva	mandibles	Zn
		claws	Zn
DIPTERA			
Contarinia oregonensis	larva	spatula	none
Delia anthracina	eggs		none
	instar 2	mouth hooks	Ca, Mn
	instar 3	cuticle	none
		mouth hooks	Ca, Mn
	instar 4	mouth hooks	Ca, Mn
Earomyia abietum	larva	mouth hooks	Ca
Earomyia barbara	larva	cuticle	Ca
		mouth hooks	Ca, Zn
HEMIPTERA			
Leptoglossus occidentalis	instar 1	proboscis	none
		claws	none
	instar 2	proboscis	Mn
		claws	none
	adult	proboscis	Mn
		claws	none
HYMENOPTERA			
Megastigmus spermatotrophus	larva	mandibles	Zn
COLEOPTERA			
Dendroctonus ponderosae	instar 3	mandibles	Zn
	instar 4	mandibles	Zn
	adult	mandibles	Zn
		claws	Zn
Enoclerus schaefferi	larva	mandibles	Zn, Mn
		claws	Zn
		anal hooks	Zn, Mn
Neacanthocinus obliquus	adult	mandibles	Mn
		claws	Mn

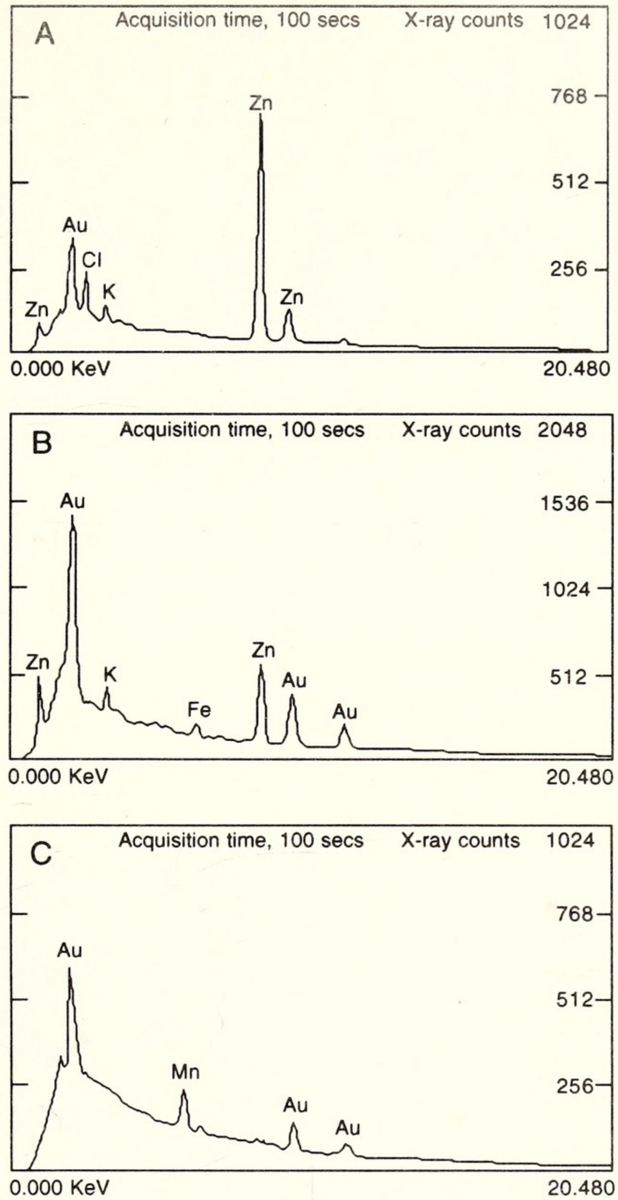

Fig. 1. Representative EDXa spectra. Gold peaks are artifacts as explained in the text. A. From the mandibular cutting edge of *B. colfaxiana*, instar 4, showing prominence of zinc peaks. A chlorine peak is also evident. B. From a claw tip of *B. colfaxiana*, instar 4, showing the presence of zinc. C. From a stylet of an *L. occidentalis* adult, showing a relatively weak manganese peak.

on conventionally prepared specimens using a JEOL JSM-35 scanning electron microscope.

RESULTS

A synopsis of metals detected is shown in Table 2. Each report of a metal finding is based on analysis of at least 3 specimens. Spectra were typically replicated three times for each sample point.

Among lepidopteran species, larvae showed a consistent pattern of zinc accumulations along the cutting edge of the mandibles and in claw tips. Zinc or other metals were not detectable in the cuticle of the body generally except in these areas. Most information on zinc distribution in the lepidopterans available to us is from *B. colfaxiana* for which we had a full range of stages. In that species, zinc is found along the cutting edge of the mandibles in all instars (Figs. 1A, 3) but it is not present in the mouth structures of the adult. We did not attempt accurate quantification but, estimated from X-ray counts for the Zn Kα peaks and comparison of X-ray images, the relative amount of zinc along the mandibular cutting edge apparently increases in each successive instar. Zinc is also precisely localized in the larval claw tips (Figs. 1B, 4). Again each instar shows relatively

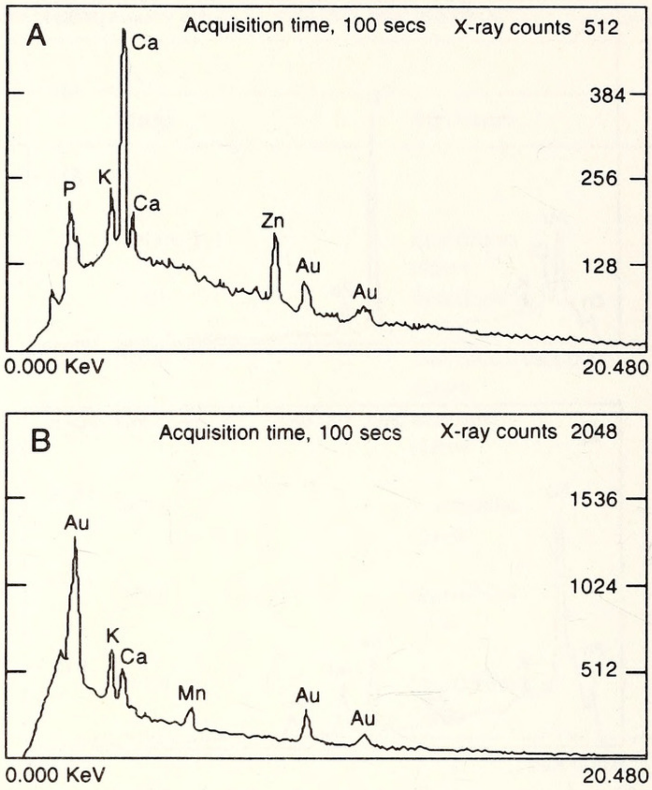

Fig. 2. Representative EDXa spectra from dipteran mouth hooks.. Gold peaks are artefacts as explained in the text. A. From the base of a mouth hook of *Earomyia barbara* showing the co-occurrence of calcium and zinc. B. From the tip of a mouth hook of *Delia anthracina* showing the co-occurrence of calcium and manganese.

greater zinc concentrations, but it is absent from adult claw tips. A chlorine peak accompanies the zinc peak in the mandibles, but chlorine is distributed more widely throughout the mandibular cuticle. Chlorine appears to be in highest concentrations along mandibular cutting edges where zinc is localized. Minor chlorine peaks are inconsistently present in claws, but not generally over the body surface. A potassium peak is common over the entire body surface. Gold peaks are, of course, artifacts of gold-coating.

Although the material available for analysis was not so extensive, the larvae of four other lepidopteran species showed a similar pattern of zinc distribution, accompanied by chlorine as described above. Zinc X-ray images show the spatial distribution of zinc in the mandibles and claw tips of the Douglas fir fall cone worm, *H. immaculella*, for example (Fig. 5, 6), and demonstrate the consistency of the pattern of metal distribution in these lepidopteran species.

Zinc was found in the small mandibles of the larvae of the hymenopteran, *M. spermatotrophus*, but X-ray maps were not successfully obtained because their small size proved difficult to work with. The larvae lack claws.

The coleopteran species did not show a consistent pattern of metal accumulation. The mountain pine beetle, *D. ponderosae*, had major accumulations of zinc in the cutting edges of the mandible of larvae and adults. Small amounts of manganese accompanied zinc in the mandibles of the larval checkered beetle, E. *schaefferi*, and zinc alone occurred in the larval claw tips. The long-horned wood borer, *N. obliquus*, had small accumulations of manganese in adult mandibles and claw tips.

The seed bug, *L. occidentalis*, had small amounts of manganese (Fig. 1C) in stylets of the second instar nymph and adult, but none was detected in the first instar nor in the claws of any of the stages examined. Where manganese occurred, it was not restricted to the tip but was distributed uniformly throughout the stylet.

Dipteran species had complex patterns of metal accumulations, but calcium was typically prominent. Calcium was found widely distributed throughout the cuticle of mouth

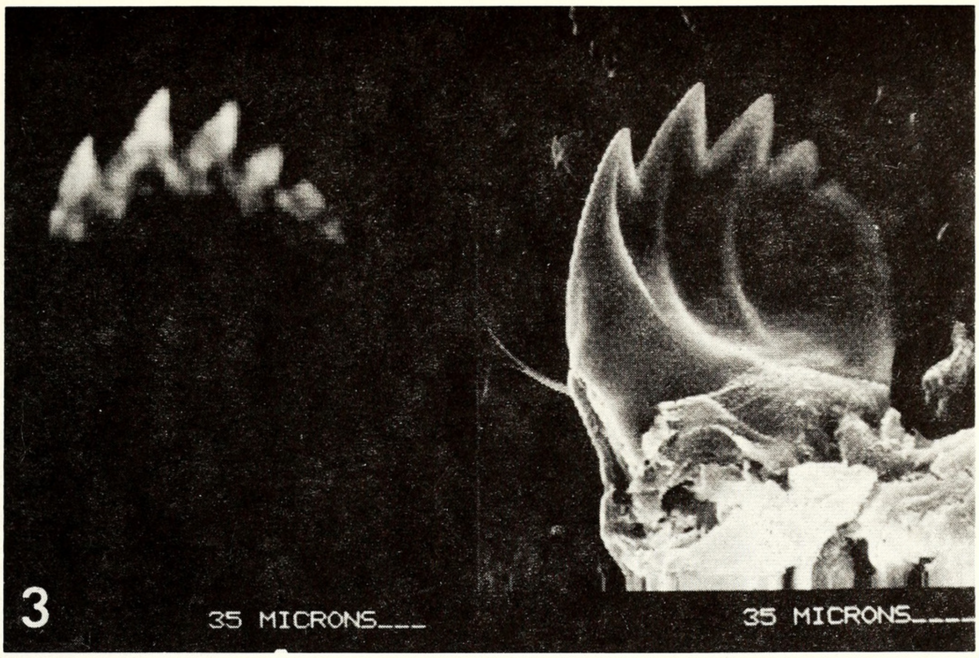

Fig. 3. SEM and zinc X-ray images of the mandible of *Barbara colfaxiana*, instar 3. Right, digitized SEM image of the left mandible, inner surface. Left, zinc X-ray image indicating the morphological distribution of zinc within that mandible

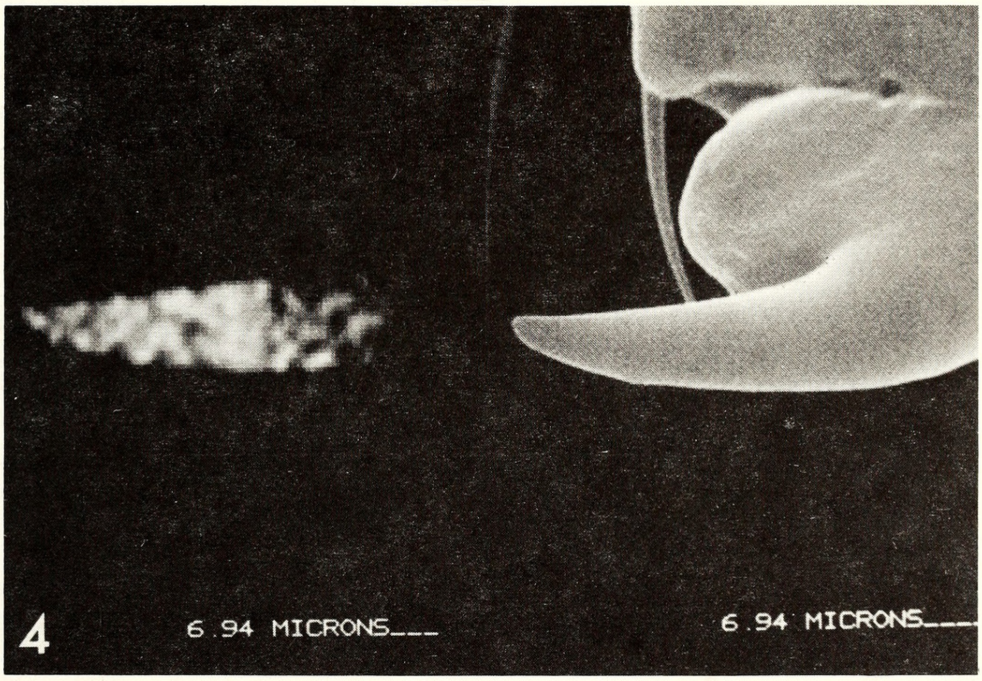

Fig. 4. SEM and zinc X-ray images of a claw of *Barbara colfaxiana*, instar 3. Right, digitized SEM image. Left, zinc X-ray image showing the distribution of zinc in the claw tip.

hooks of all species, with the exception of *C. oregonensis*. Additionally, zinc was detected together with calcium in the mouth hooks of *E. barbara* (Fig 2A) and manganese along with calcium in *D. anthracina* (Fig. 2B). Low levels of calcium were also present throughout much of the larval cuticle of *E. barbara*.

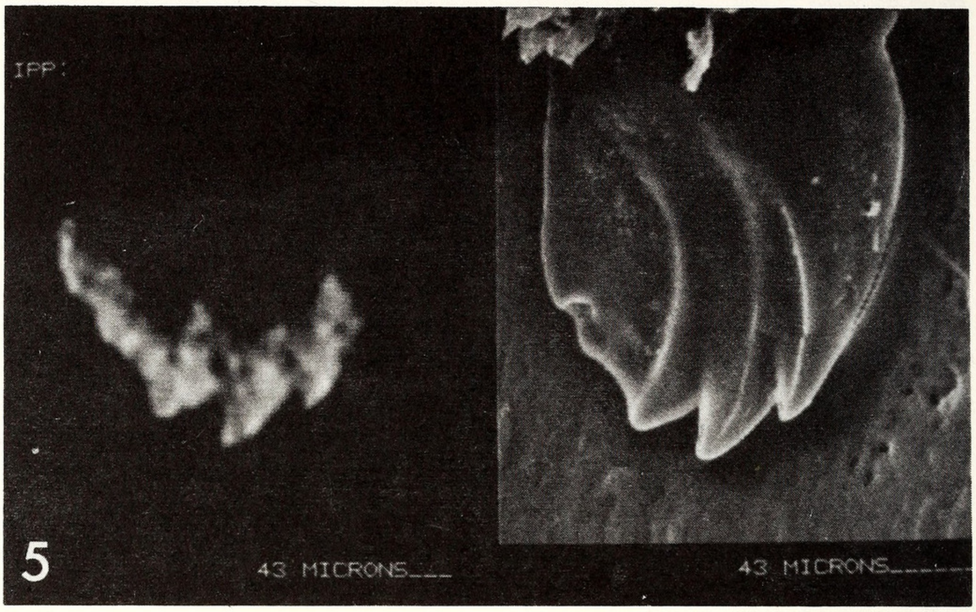

Fig. 5. SEM and zinc X-ray images of the mandible of a larval *Holocera immaculella*. Right, SEM image, left mandible, inner face. Left, zinc X-ray image showing the distribution of zinc in that mandible.

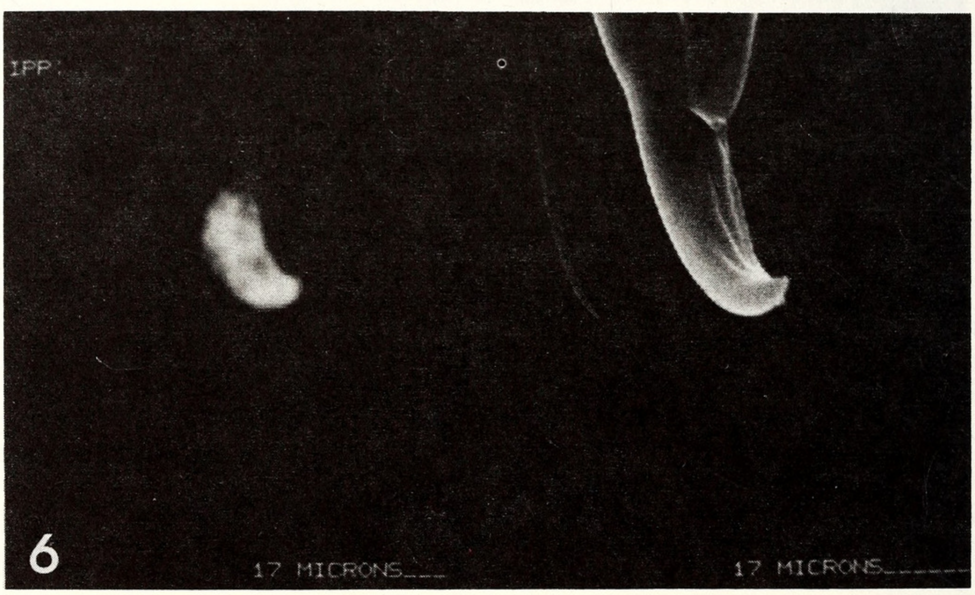

Fig. 6. SEM and zinc X-ray images of a claw of a larval *Holocera immaculella*. Right, SEM image of a claw. Left, zinc X-ray image showing the distribution of zinc in the claw tip.

DISCUSSION

In discussing cuticular metal hardening in arthropods, we are considering non-crystalline, amorphous metal deposits within cuticular substance (Hillerton and Vincent, 1982; Schofield and Lefevre, 1989), a situation distinct from better known hardening mechanisms based on highly ordered biominerals, as in ferric mineral capping of chiton or fish teeth (Sparks *et al.*, 1990).

Cuticular metal hardening in insects appears to have at least two aspects, probably related to differing biomechanical mechanisms. In one case, much metal is deposited in association with a well-defined area subject to wear; for example, zinc deposits along the cutting edge of coleopteran mandibles. In the other situation, relatively little metal is distributed uniformly throughout a structure; for example, manganese in the proboscis of the seed bug where there is too little metal to believe that its mere physical presence confers hardness. However, in a situation like this, a little metal could significantly affect stiffness or some other biomechanical property via promotion of secondary bonding of cuticular proteins (Hillerton and Vincent, 1982; Schofield and Lefevre, 1989). In our view, which of these alternative metal-based adaptations is employed appears to be related to the feeding biology of the insect. In some species, two metals co-occur in a structure, suggesting to us cases where the function and biomechanics of the structure require simultaneous employment of both adaptations.

Our evidence shows that zinc concentrations along mandibular cutting edges and in claw tips are common in herbivorous forest insects, particularly where feeding requires mining through bark or cone scales or prolonged tunneling within inner bark or developing cones (Ruth, 1980; Wood and Van Sickle, 1987). The lepidopteran species examined share a similar feeding strategy, mining as larvae into cones to feed on developing seeds. Zinc is prominent in mandibles and claws of adult and larval mountain pine beetles, *D. ponderosae*, which tunnel through bark as adults and as larvae mine tissues of inner bark. Zinc is found in the mandibles and claws of the checkered beetle, *E. schaefferi*, which, although a predator upon *B. colfaxiana* larvae, mines through bark or cones to find them (Moeck and Safranyik, 1984). The Douglas fir seed wasp, *M. spermatotrophus*, oviposits through cone scales into developing seeds on which its larvae feed (Ruth, 1980; Wood and Van Sickle, 1987) using zinc-hardened mandibles.

Development of toughness in plants is a defensive adaptation known to affect morphology, feeding behaviour and distribution patterns of some herbivorous insects (Feeny, 1970; Djamin and Pathak, 1979; Raupp, 1985). Such relationships are likely to exist in the mouthpart and claw tip adaptations of herbivorous forest insects, particularly cone and seed insects.

Zinc is the metal typically accumulated by terrestrial arthropods for cuticular hardening of the kind described above. For example, zinc occurs in the cheliceral fangs of several spiders (Schofield and Lefevre, 1989), in the tips of chelicerae and pedipalps of a scorpion and in the mouthparts of a mite (Fontaine and Pedersen, unpublished observations). Metals accumulated by aquatic organisms for cuticular hardening are more diverse. Some examples include zinc or copper in marine polychaete jaws (Gibbs and Bryan, 1980), silicon in chaetognath teeth (Bone *et al.* 1983), and silicon and zinc in copepod mandibles (Perry *et al.*, 1983).

The alternative pattern for metal deposition is that the metal occurs in small quantities distributed diffusely within a structure. As suggested above, metals in these situations may have biomechanical roles (e.g., stiffness, resistance to fracture) via metal biochemistry but their function is unlikely to be metal hardening as such. Manganese has this sort of distribution in the species we surveyed. In contrast to the herbivorous insects, the manganese-accumulating species do not mine or chew continually and employ diverse feeding strategies as in, for example, the seed bug, *L. occidentalis*, and the long-horned wood borer, *N. obliquus*. In the seed bug, manganese occurred in the stylets of the second instar and adult which feed on cone seeds by inserting the stylet through the tough seed coat, enzymatically digesting and then ingesting the endosperm (Ruth, 1980). The first instar nymph feeds on foliage rather than on seeds and lacked any metal in the stylets. In contrast to the herbivorous chewing insects, mouthparts like these must have different biomechanical requirements which, in our opinion, are reflected in the metal distribution pattern.

Major amounts of calcium deposits in insect cuticle seem to be restricted to dipteran species, according to our results and others (Grodowitz and Broce, 1983; Roseland *et al*, 1985). Calcium may be functionally analogous to manganese since it also occurs in small quantities with a diffuse distribution. However, it is interesting to note that calcium co-occurs with manganese in *D. anthracina* mouth hooks and with zinc in *E. barbara* mouth hooks.

Co-occurring metals (calcium/manganese in *D. anthracina*; calcium/zinc in *Earomyia barbara*; manganese/zinc in *Enoclerus schaefferi*) suggest structures where interaction of two distinct biomechanical mechanisms is required and is accomplished by using different metal hardening processes (Schofield et al., 1988; Schofield and Lefevre, 1989). Once again, these differences may reflect adaptive requirements based on feeding or dietary differences, such as relative abrasiveness of food. Among the fir cone maggots, for example, *Earomyia barbara* (calcium/zinc) uses its mouth hooks to feed on Douglas fir cones which are tougher than the balsam fir cones fed on by *E. abietum* (calcium alone) (D. S. Ruth, personal communication).

Hillerton, Robertson and Vincent (1984) considered the occurrence of zinc or manganese in mandibles of coleopteran species to be correlated with taxonomy and to be a reflection of evolutionary history of the group, despite some paradoxes of metal distribution within some sub-taxa. In our view, the metal or metals that occur in a species are more likely to be correlated with biomechanical adaptations of feeding structures. Since species groups within a family often employ a similar feeding strategy, they are likely to share similar biomechanical adaptations and similar uses for a metal. By contrast, calcium accumulation is apparently unique to Diptera and in that taxon may well be the result of phylogenetic conservatism.

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