tained by 2 of the 5 EE clones. The EE_3 and EE_5 clones in question nevertheless produced considerably more nymphs than any of the maternal C aphids of similar weight (tables 1 and 2). The mean weights of young adult aphids of each clone after a further 4 generations on the IS 809 sorghum are given in table 3. The relative performance of these G_5 clones (fig. III) is the same as that of the G_1 aphids (fig. II). However, less variability occurred between clones of the same crosses, and the performance of all the clones was some 50% poorer than that of the G_1 aphids. Because the mothers of the latter had developed on susceptible barley (on which they grew twice as big and produced 70–80 nymphs) it is possible that nutritional factors passed transovarially to the G_1 aphids enabled them to grow substantially better than the later generations on the IS 809 sorghum.

As summarized in figure IV, the results indicate that it is the maternal biotype which determines if an individual *S. graminum* can overcome plant resistance. This pattern of inheritance cannot be explained in Mendelian terms. Possible explanations for the phenomenon include extra-nuclear inheritance and parthenogenetic development of the eggs. While parthenogenesis is the mode of reproduction by viviparous aphids, it is unlikely that it takes place in the winter eggs of aphids, because the few eggs that virgin oviparae may deposit do not turn black (as do the majority of the eggs deposited by mated oviparae) and they shrivel when incubated. On the other hand, cytoplasmic transmission of symbiotic microorganisms from oviparae to their eggs is well documented[8], and extra-nuclear inheritance has been shown to affect the fitness of insect populations in various ways[9].

The symbiotes of aphids appear to play important roles in the nutrition of their hosts by providing them with some essential nutrients[10–12]. That the symbiotes may also provide aphids with certain enzymes that are able to hydrolyze plant matrix polysaccharides has been inferred for *S. graminum*. These enzymes have been surmized to facilitate inter-cellular penetration of plant tissues by the aphid's stylets, with an increase in enzyme activity in biotype E being correlated with the ability to overcome this host plant barrier in biotype C-resistant sorghum plants[13, 14]. The breakdown products of these hydrolyses may also make the plant more acceptable to the aphids[15]. Such attributes have been invoked to explain the ability of *S. graminum* to overcome resistance in sorghum, and may have been acquired as a result of the rapid generation turn-over of its symbiotic microorganisms[16]. The maternally-related performance of the hybrids may best be explained in terms of the extra-nuclear inheritance by the aphids of symbiotes from their mothers.

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Chronic mercury vapor poisoning of aphids

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Summary. Exposure of green peach aphids, *Myzus persicae* (Sulz.), to an atmosphere containing mercury vapor resulted in a curtailment of embryogenesis and larviposition by adults, and in the development by larvae and adults of a cuticular darkening of their legs, head capsule, antennae, cornicles and cauda. Mortality of affected larvae resulted from molting difficulties, particularly by last-instar alatiform female and male larvae. Greenbugs, *Schizaphis graminum* (Rond.), and pea aphids, *Acyrthosiphon pisum* (Harr.), responded to mercury vapor exposure in similar ways.

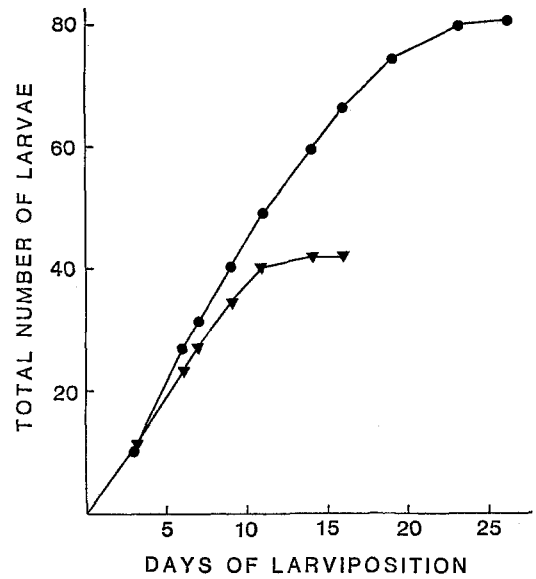
Key words. Aphids; green peach aphids; *Myzus persicae;* greenbug; *Schizaphis graminum;* pea aphid; *Acyrthosiphon pisum;* mercury vapor; contamination; pigmentation; reproduction.

In a clonal culture of the green peach aphid (a biotype of *Myzus persicae* (Sulz.) from Yakima, Washington State) maintained in one of our temperature- and light-controlled cabinets, we detected several aphids of apparently another species. These aphids were of similar shape to the *M. persicae* but their bodies were conspicuously yellowish, and their antennae, legs, cornicles and cauda were dark grey or black, and some had a dusky head capsule. The presence of these aphids was at first ascribed to contamination by stray aphids of the greenhouse-grown radish seedlings on which the culture was maintained. However, after the establishment of a new clonal culture of the *M. persicae,* some of the unusual aphids were again found together with normal aphids on each of the radish seedlings placed in the same cabinet. This was the case despite close scrutiny of the plants for possible strays, the plants' individual confinement within screen-topped plastic cages, and the absence of any other aphid/plant cultures in this cabinet.

Careful inspection of the affected cabinet revealed a few small beads of mercury in crevices on its floor. These stemmed from a broken thermometer that had otherwise been removed from the cabinet a week before the first observation of the unusual aphids. Since the cabinet, a modified 280-l refrigerator, was almost airtight, it appeared possible that a mercury vapor build-up within it could have been responsible for the observed changes in the aphids.

The present paper describes some of the experiments which tested this possibility and evaluated the effects of exposing various morphs of *M. persicae* and 2 other aphid species to mercury vapor at levels that could arise under accidental circumstances such as described above.

Methods and results. When *M. persicae* larvae were allowed to develop from birth on radish seedlings confined in a 9-l desiccator jar containing a single bead (0.2 g) of mercury, the majority of the larvae developed the unusual characters. Many of the aphids died before reaching adulthood and most of those that became adult were sterile. The radish seedlings were also severely affected by this treatment. There was an early yellowing of their cotyledons, a curling of the cotyledons and of the primary



Cumulative total numbers of larvae deposited by apterous virginoparous green peach aphids exposed to mercury vapor (▼) and by control aphids (●).

leaves, and some of the seedlings died within 7–10 days of enclosure in the jar.

In order to test whether the mercury vapor affects the aphids directly or indirectly via their host plants, adult apterous viviparae were exposed to a mercury-saturated atmosphere while they were removed from their host plants for a few hours on each of 10 successive days. Subsequent to each exposure, the aphids were allowed to feed and larviposit on a succession of radish seedlings unexposed to mercury.

Most of the larvae born to these aphids after 4 or more such exposures showed some degree of abnormality. However, the symptoms were generally not as intense as in the previous examples, presumably because of the shorter daily exposure of the adults and because the larvae were not also exposed to the mercury vapor.

In order to expose both the adult aphids and their larvae to a lower concentration of mercury vapor, the insects and plants were confined continuously within a larger container (a 35-l aquarium tank, also with a 0.2-g bead of mercury) whose glass lid was opened once a day. Although the latter system did not permit quantification of the mercury exposure, it proved to be the most practical way of exposing a larger number of aphids and plants to chronic levels of mercury-vapor poisoning such as they may have experienced in the accidentally contaminated insect/plant-growth cabinet.

To monitor the effect on the aphids of this level of exposure, young adult apterous viviparae were confined (one per radish seedling) in the mercury aquarium and transferred to fresh seedlings every 2–3 days until the insects died. The larvae deposited during each successive birth period were counted, returned to the aquarium on the seedling on which they had been born, and examined periodically during their development to adulthood. Aphid-free radish seedlings were also held in the aquarium for 2 weeks, to ascertain the effect of the mercury vapor on the plants. Control plants and aphids were maintained simultaneously in a comparable mercury-free environment.

In the aquarium containing the mercury, most of the early-born larvae in this succession had no unusual features and developed into normal reproductive apterous viviparae – the morph expected under the experimental long-day photoperiodic conditions. However, after 2–4 days of reproduction by the initial adults, progressively more of the larvae not only had dark antennae at birth but, in their subsequent development in the mercury aquarium, their legs, cornicles, and cauda became grey or black. The affected larvae developed more slowly and were smaller at adulthood than normal apterous viviparae of *M. persicae*. The most pigmented adults either did not produce any larvae, although they were replete with embryos, or they deposited only a few larvae some of which were stillborn.

The figure compares the reproductive performance of 12 mercury-exposed aphids to that of 12 control aphids. The fecundity of the aphids was similar during the first 8–9 days of larviposition. Thereafter, however, the mercury-exposed adults deposited only a few more larvae, were almost completely devoid of embryos, and developed the translucent yellow appearance of normal post-reproductive apterous viviparae of *M. persicae*. It appears therefore that the mercury-exposed aphids were able to mature their initial complement of embryos, but that additional embryogenesis in these aphids was curtailed well before their death 16–19 days into adulthood. The control adults, on the other hand, continued at their initial rate of larviposition for another 2 weeks, produced twice as many (81 versus 42) larvae, and lived almost twice as long, as the mercury-exposed adults. The wet and dry weights of radish seedlings maintained for 2 weeks in the mercury aquarium were 70–80% of those of the control seedlings.

When presumptive alate viviparous and male larvae (produced by adults reared under a short-day regime) were exposed to mercury vapor from birth, they not only developed dark antennae, legs, cornicles and cauda, but the wing buds in the 4th larval instar were distinctly darker than those of the controls. A further anomaly arose when these alatiform larvae molted. Some of those that successfully attained adulthood showed various degrees of crumpling of the wings and some had fluid-filled vesicles in parts of the wings. Other larvae died during the molt, because they were unable to extract themselves from the last larval cuticle. As was the case with the pigmented apterous adult viviparae, some of the affected alate viviparae either produced no larvae or only deposited some aborted embryos.

Exposure to mercury vapor of adult viviparae of the greenbug, *Schizaphis graminum* (Rond.), and of the pea aphid, *Acyrthosiphon pisum* (Harr.), resulted in pigmentation changes similar to those in *M. persicae*. With *S. graminum*, premature death of the adults and pre-adult mortality of most of their larvae indicated a higher sensitivity of this species to similar levels of mercury poisoning.

On molting, the dark pigmentation in the insects' extremities remained in the otherwise white exuviae. Since it was absent in newly molted aphids, one may conclude that the pigmentation is cuticular rather than epidermal.

Discussion. Elemental mercury and many compounds of mercury have a high volatility and are known to be highly toxic to living organisms in their vapor phase as well as in their liquid/solid or dissolved states[1–4]. However, to our knowledge, no records pertain to the accidental or experimental exposure of insects to mercury vapor. The changes thereby induced in aphids appear to be similar to some of the effects on locusts of $HgCl_2$ injections[5] and on *Drosophila* of organic mercury ingestion[6].

Whether chromosomal abnormalities such as reported in the *Drosophila* study are involved in the cessation of reproduction by the mercury vapor-exposed aphids requires investigation, in light of the sensitivity of the oocyte chromosomes of aphids to chemical changes[7]. The present report shows that chronic mercury vapor poisoning of organisms may occur under circumstances that can realistically be encountered in the laboratory. Such poisoning may have subtle effects that could remain undetected or be ascribed to other causes. If this paper stimulates an awareness, or acts as a reminder, of the toxicity of mercury vapor under laboratory conditions[8–10], much will have been achieved beyond the description of the phenomenon with aphids.

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Detection of protease inhibitors in the hemolymph of resistant *Anticarsia gemmatalis* which are inhibitory to the entomopathogenic fungus, *Nomuraea rileyi*

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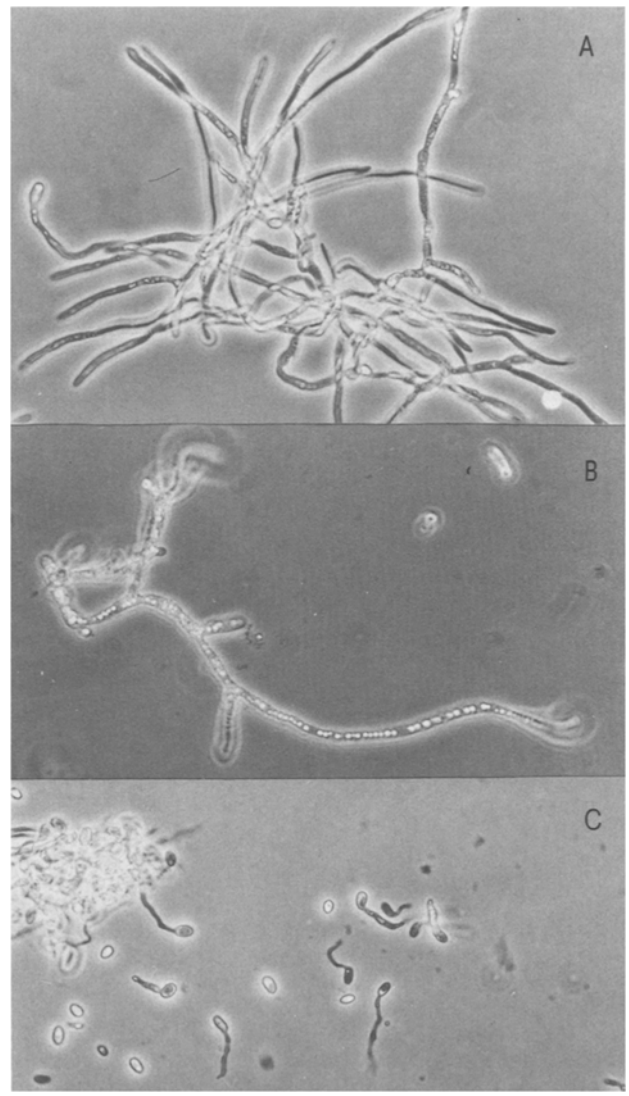
Summary. A complex of protease inhibitor activities has been detected in the hemolymph of the 6th instar *Anticarsia gemmatalis* larvae that are resistant to infection by the fungus *Nomuraea rileyi*. A site-specific serine protease inhibitor extracted from *A. gemmatalis* hemolymph inhibits both the germination of *N. rileyi* conidia and subsequent germ tube development.
Key words. Protease inhibitor; insect immunity; *Anticarsia gemmatalis; Nomuraea rileyi.*

The entomopathogenic fungus, *Nomuraea rileyi,* is recognized as an important naturally occurring biological control agent of a variety of noctuid pest defoliators[1]. This species is comprised of numerous pathotypes having distinct biological properties[2,3]. Many of the *N. rileyi* pathotypes are highly virulent to larvae of the cabbage looper, *Trichoplusia ni,* whereas only a few pathotypes (i.e. F178-6) have been shown to be virulent to the velvetbean caterpillar, *Anticarsia gemmatalis*. As this host insect matures it becomes resistant to those *N. rileyi* pathotypes that are virulent to younger *A. gemmatalis* larvae (1st-4th instar). The mechanisms responsible for the resistance expressed by mature *A. gemmatalis* larvae are poorly understood. Bioassays involving the injection of vegetative cells of various *N. rileyi* pathotypes into the hemocoel of late instar *T. ni* and *A. gemmatalis* produce a differential mortality response similar to that achieved with topical application[4]. These findings suggest that the resistance of late instar *A. gemmatalis* to *N. rileyi* is due in part to an internal defense system.

Recently, it has been proposed that protease inhibitors, detected in hemolymph samples from various invertebrates, play a defensive role against invading microorganisms[5-12]. Entomopathogenic fungi, such as *N. rileyi,* are believed to require a complex of hydrolases (protease, lipase, chitinase) for both penetration and colonization of host insect tissues. Inhibition of these hydrolases could abort the infection process and confer resistance to insect species possessing such protease inhibitory activities. The relative numbers and specific activities of hydrolase inhibitors detected in invertebrates is variable. In the silkworm, *Bombyx mori,* a series of inhibitors ranging from 7000 to 60,000 daltons, which have either antitrypsin or antichymotrypsin activities, have been isolated and reported to be active against commercially available fungal proteases[13-15]. Low molecular weight inhibitors detected in waxmoth larvae, *Galleria mellonella,* were reported to be active against a toxic protease produced by the entomopathogenic fungus, *Metarhizium anisopliae*[5-7]. Hall and Soderhall[16,17] extracted a protease inhibitor from both blood cells and from the cuticle of the crayfish, *Astacus astacus,* which inhibits the protease activity of the fungal entomopathogen *Aphanomyces astaci*. Recently, high molecular weight protease inhibitors possessing properties similar to the α-macroglobulins have been detected in the hemolymph of Crustaceae[18].

In this paper we demonstrate the presence of a protease inhibitor complex present in resistant late instar *A. gemmatalis* larvae that is not detected in the early instars of *A. gemmatalis* and *T. ni* larvae. Furthermore, we show that chromatographic fractions of *A. gemmatalis* hemolymph containing protease inhibitory activity are deleterious to the growth and development of the fungus *N. rileyi*.



Light micrographs of preparations of germinating *Nomuraeae rileyi* conidia that were been incubated in (A) one half strength SMY broth (× 1700); (B) SMY broth cell-free *Anticarsia gemmatalis* hemolymph (1:1 concentrations, × 1600) and (C) SMY broth: insect protease inhibitor (15.2 inhibitor units/μl, × 1600).