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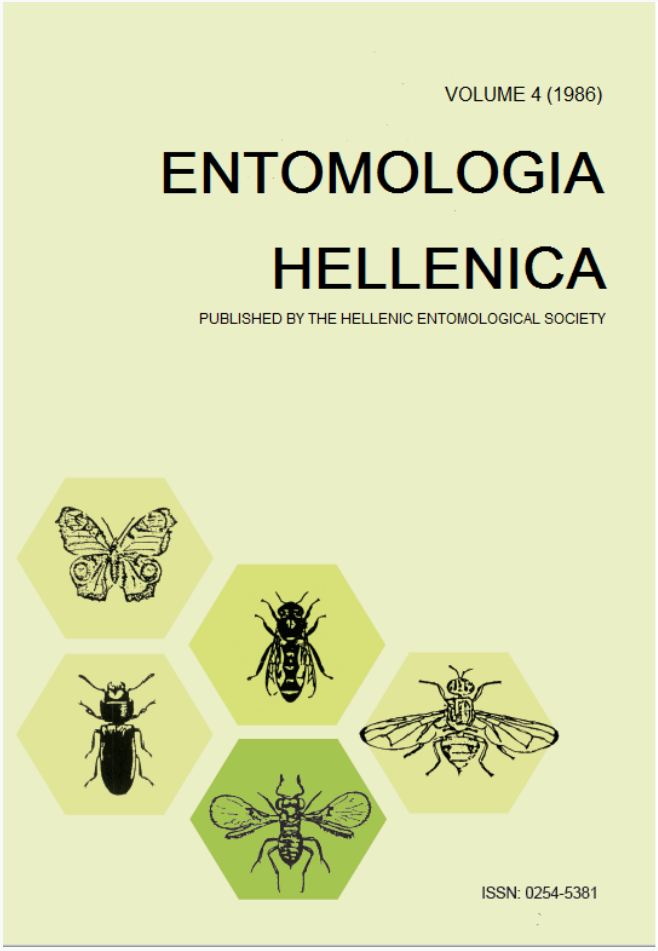

Προκαταρκτική Αναζήτηση Ιών σε Πληθυσμούς Dacus oleae Gmel. της Βόρειας Ελλάδας

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Preliminary Search for a Virus in *Dacus oleae* Gmel. Populations in Northern Greece[1]

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ABSTRACT

A large number of larvae of *Dacus oleae* were collected from infested olives in Northern Greece, and a small proportion of these were found to be dead. Adult flies were caught in McPhail traps at the same locations. The larvae and adults were fractionated by a series of steps designed to identify occluded and nonoccluded viruses. Virus-like particles were identified in small amounts only in the dead larvae.

Introduction

The olive fruit fly *Dacus oleae* (Gmelin) (Diptera, Tephritidae) is a major pest of the olive causing early fruit drop, "sting" damage to table olives and substantial decreases in the quantity and quality of oil. Considerable resources are currently directed towards controlling this pest; the procedures employed include chemical control and integrated methods which employ the synergistic effects of distinct agents. Virological control has been successful with several other pest species of insects. Viruses are reasonably specific and target insects do not appear to become resistant to these agents (Entwistle 1983, Maramorosch and Sherman 1985). Therefore we decided to investigate populations of *D. oleae* with the objective of isolating viruses which might prove to be useful field control agents.

Materials and Methods

Infested olives were collected from July to December 1984 in areas of central Northern Greece selected for heavy infestation and the absence of chemical insecticides (see Fig. 1). Olives were carefully dissected on the day of collection and larvae and pupae were removed and stored at –28°C. Adults were caught in McPhail traps baited with protein hydrolysate and borax water solutions.

All fractionation procedures on the immature and adult *D. oleae* were performed on ice or at 4°C. Aliquots of larvae, pupae or flies were macerated

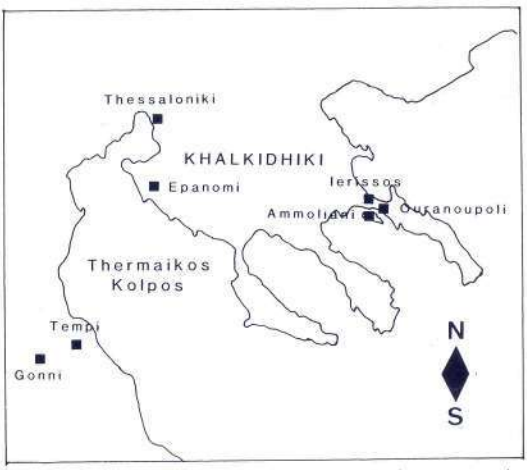

FIG. Black quadrangles represent the areas in central Northern Greece, where samples of flies, larvae and pupae were obtained.

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in a Colworth Stomacher/Lab-Blender in 10-15ml of 50mM Tris-HCl pH 7.2 for 5-10min. The homogenate was filtered through two layers of butter muslin and centrifuged for 10 min at 3000g. A portion of the pellet was retained for light microscopy after resuspension in 50mM Tris-HCl pH 7.2. The remainder was overlaid onto a 10-50% discontinuous sucrose gradient and centrifuged for 20min at 12,500g. Bands were collected and examined by light and electron microscopy. The 3000g supernatant was centrifuged for 30min at 12,000g, the pellet was retained and the supernatant was centrifuged for 2hrs at 107,000g over a pad of 15% sucrose in 50mM Tris HCl pH 7.2. The resuspended pellet was overlaid on a linear 10-50% (w/w) sucrose gradient and centrifuged for 1.5hr at 150,000g. The gradient was divided into 6 fractions and each was diluted in 50mM Tris-HCl pH 7.2 and centrifuged for 3hr at 88,000g. The pellets were resuspended and examined by negative staining and electron microscopy. The resuspended pellets were further purified on 34% (w/w) initial concentration CsCl in 10mM phosphate buffer pH 7.2 centrifuged at 150,000g for 16hrs.

Material in pellets from the initial stages of the purification were examined for the presence of occluded viruses, by staining with Giemsa and Naphthalene Black 12B followed by light microscopy. The pelleted material from gradients was stained with 2% uranyl acetate in distilled water and examined in a Jeol JEM-100S electron microscope at 100KV.

For detection of nucleic acids of possible viral origin, various fractions were proteinase K-treated and phenol-extracted as described by Reavy and Moore (1981a). Nucleic acids were analysed by agarose electrophoresis and ethidium bromide staining (Reavy and Moore 1981b). An extraction procedure designed to identify double-stranded RNA was also used. 120mg of dead melanised larvae were homogenized for 30min in STE (0.1M NaCl, 0.05M Tris, 0.001M EDTA pH 7.0) with a pestle and mortar. The extract was treated with phenol/TE (10mM Tris, 1mM EDTA, pH 7.4) for 30min at ambient temperature. After centrifugation, the aqueous phase was extracted with ether, made 15% with ethanol and 0.25 g Cellex NI (Bio-Rad) was added per 20ml. After 10min agitation on ice, the mixture was centrifuged for 10min at 3,500g. The cellulose was resuspended in 0.25 volumes of STE/15% ethanol and placed in a pasteur pipette, where it was washed with STE/ethanol until zero absorption at A_{260}. Double-stranded RNA was eluted with STE.

Due to the unavailability of *D. oleae* larvae or flies, an attempt was made to propagate any virus-like material in injected *Galleria mellonella* larvae and infected *Drosophila melanogaster* culture cells (Moore et al. 1980).

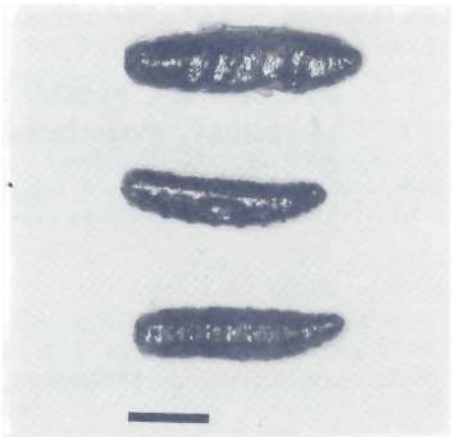

FIG. 2. Melanized dead larvae of *D. oleae* collected by dissection of infested olives. Similar larvae were used for the isolation of particles (see text). Bar = 2mm.

Results and Discussion

A total of 4.5g of adult flies were caught in McPhail traps and 8.5g of healthy larvae and pupae were dissected from approximately 95Kg of olives. A surprisingly large amount of dead and melanized larvae was also found in the olives – a total of 0.45g. The majority of these larvae were mature (see Fig. 2) and as the collection sites had been selected for low incidence of pesticide use the mortalities could not readily be explained. 0.25-2g of larvae, pupae or flies were macerated in buffer and after staining the macerates were subjected to further fractionation. Any occluded viruses are normally separated in the first low speed pelleting step and can be more readily identified by analysis on a 10-50% (w/w) discontinuous gradient. Conventional staining procedures followed by light microscopy should show the presence of the occluded viruses such as nuclear and cytoplasmic polyhedrosis viruses which have been demonstrated to be efficient biological control agents when used against some pest insect species. We were unable to identify viruses of this type in any of the samples, including the fractionated field-collected dead larvae.

A large number of virus types have been reported to be associated with insects, and the further gradient fractionation steps were designed to purify viruses such as the small RNA viruses, reoviruses, densonucleoviruses, iridoviruses, etc. No small virus particles were detected in the healthy larvae or adult flies.

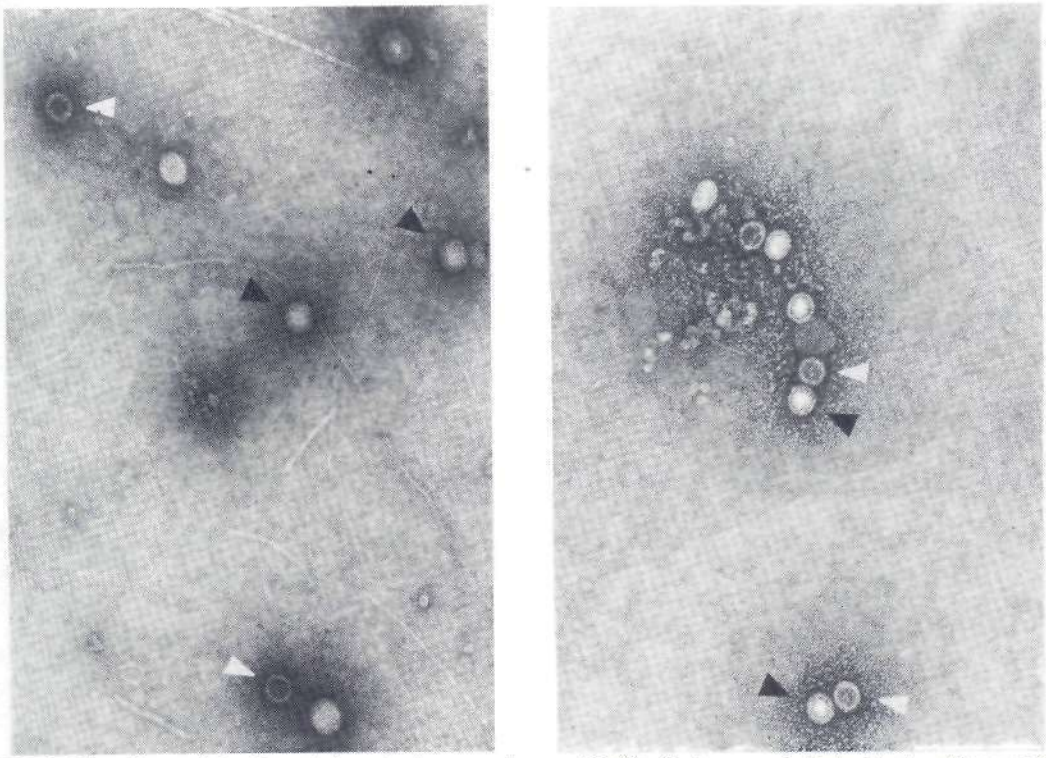

FIG. 3. Virus-like particles. Negatively stained preparations; x 125,000. Black arrows indicate intact particles; white arrows indicate "empty" ones.

When material from the field-collected dead larvae was fractionated on the 10-50% (w/v) sucrose gradient and fractions examined by electron microscopy, virus-like particles were detected (see Fig. 3). The particles had a diameter of $\simeq$ 35nm and some were apparently empty as demonstrated by the penetration of stains. Unfortunately there was inadequate material to perform further detailed characterisation. Attempts to obtain enough nucleic acid to further characterise the virus-particles proved unsuccessful as did the extraction of double-stranded RNA from the dead larvae. The latter method is normally successful with material infected with double-stranded RNA viruses or for isolating the replicative form RNA from single-stranded RNA viruses. Attempts to introduce the virus-like particles into *Drosophila melanogaster* tissue culture cells and *Galleria mellonella* larvae also proved unsuccessful. These two systems were chosen because they can successfully support many virus types. In view of the relatively small amount of material available it was decided to retain the rest of the samples until a successful *D. oleae* colony was established when attempts to infect the homologous insect will be made.

While the small virus-like particles were the only possible pathogens identified in the dead larvae (no fungi or bacteria were apparent) it seems unlikely that the relatively small number of particles identified could be the sole cause of death. However, if these "viruses" can be propagated in *D. oleae* or a tissue culture system derived from them, they may prove to be a biological control agent. We are continuing in our efforts to investigate if these particles can be effective in controlling *D. oleae*, and to find alternative viruses to suppress this serious pest. Bergoin et al. (1983) identified two types of virus in natural populations of *D. oleae*, one of which was a reovirus, but neither appeared to be pathogenic.

References

Bergoin, M., M. Anagnou-Veroniki, J.C. Veyrunes and G. Croizier. 1983. Communication preliminaire, 16e Reunion Annuelle de la Société de Pathologie des In-

vertebres (SIP). Cornell University, Ithaca, N.Y.
Entwistle, P.F. 1983. Control of insects by virus diseases. Commonwealth Institute of Biological Control. Biocontrol News and Information. 4,3: 203-226.
Maramorosch, K. and K.E. Sherman (eds). 1985. Viral Insecticides For Biological Control. Academic Press.
Moore, N.F., A. Kearns and J.S.K. Pullin. 1980. Characterization of cricket paralysis virus-induced polypeptides in *Drosophila* cells. J. Virol. 33: 1-9.
Reavy, B. and N.F. Moore. 1981a. In vitro translation of cricket paralysis virus RNA. Arch. Virol. 67: 175-180.
Reavy, B. and N.F. Moore. 1981b. Cell-free translation of cricket paralysis virus RNA: analysis of the synthesis and processing of virus-specified proteins. J. Gen. Virol. 55: 429-438.

KEY WORDS: *Dacus oleae*, Virus, Olive tree

Προκαταρκτική Αναζήτηση Ιών σε Πληθυσμούς *Dacus oleae* Gmel. της Βόρειας Ελλάδας

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ΠΕΡΙΛΗΨΗ

Στην περίοδο από Ιούλιο έως Δεκέμβριο 1984 συλλέχθησαν 4.5 g ακμαίων, 8.5 g υγιών προνυμφών και 0.45 g νεκρών προνυμφών του εντόμου *Dacus oleae* Gmel. από περιοχές της Βόρειας Ελλάδας, που χαρακτηρίζονταν από βαριά προσβολή των ελαιοδένδρων από δάκο, στα οποία δεν εφαρμόστηκε χημική καταπολέμηση. Τα δείγματα εξετάσθηκαν για εγκλεισμένους και μη εγκλεισμένους ιούς με τη χρησιμοποίηση φυγοκεντρήσεων, οπτικού και ηλεκτρονικού μικροσκόπιου, ανάλυσης νουκλεϊνικών οξέων και πειράματα μολυσματικότητας. Στα δείγματα των νεκρών προνυμφών, και σε αντίθεση με εκείνα των υγιών προνυμφών και των ακμαίων, εντοπίσθηκαν και απομονώθηκαν ιόμορφα σωμάτια. Τα σωμάτια αυτά είχαν διάμετρο περίπου 35 nm και μερικά ήταν άδεια, όπως φάνηκε από τη διείσδυση της χρωστικής κατά την αρνητική χρώση. Δεν κατέστη δυνατός ο παραπέρα χαρακτηρισμός των «ιοσωματίων» για το λόγο έλλειψης αρκετής ποσότητας δείγματος, ενώ προσπάθειες πολλαπλασιασμού τους σε προνύμφες του λεπιδόπτερου *Galleria mellonella* και σε καλλιέργειες κυττάρων *Drosophila melanogaster* αποδείχθησαν ανεπιτυχείς. Αν και τα μικρά ιόμορφα σωμάτια ήταν το μοναδικό πιθανό παθογόνο αίτιο που αναγνωρίστηκε στις νεκρές προνύμφες, φαίνεται κάπως απίθανο να αποτελούν και το μοναδικό αίτιο του θανάτου για το λόγο του σχετικά μικρού αριθμού τους. Πάντως αν καταστεί δυνατό να πολλαπλασιασθούν οι «ιοί» αυτοί σε εκτροφές του δάκου της ελιάς ή σε καλλιέργειες κυττάρων ιστών του ίδιου εντόμου, ίσως να αποτελέσουν στο μέλλον ένα βιολογικό μέσο καταπολέμησής του.