AN ABSTRACT OF THE THESIS OF

Becky L. Fichter for the degree of Doctor of Philosophy in Entomology presented on April 23, 1984.

Title: Arboreal Arthropod Predation on Early Instar Douglas-fir Tussock Moth

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William P. Stephen

Loss of early instar Douglas-fir tussock moth (Orgyia pseudotsugata McDunnough) (DFTM) has been found to constitute 66-92% of intra-generation mortality and to be a key factor in inter-generation population change. This death has been attributed to dispersal and to arthropod predation, two factors previously judged more important to an endemic than an outbreak population. Polyphagous arthropod predators are abundant in the forest canopy but their predaceous habits are difficult to document or quantify. The purpose of the study was to develop and test a serological assay, ELISA or enzyme-linked immunosorbent assay, for use as an indirect test of predation.

Development of this assay involved production of an antiserum reactive with DFTM but not reactive with material from any coexisting lepidopteran larvae. Two-dimensional immunoelectrophoresis was used to select a minimally cross-reactive fraction of DFTM hemolymph as the antigen source so that a positive response from a field-collected predator would correlate unambiguously with predation on DFTM.

Feeding trials using Podisus maculiventris Say

(Hemiptera, Pentatomidae) and representative arboreal spiders established the rate of degredation of DFTM antigens ingested by these predators. An arbitrary threshold for deciding which specimens would be considered positive was established as the 95% confidence interval above the mean of controls. Half of the Podisus retained reactivity for 3 days at a constant 24°C. Eighty percent of trial-fed spiders were positive in ELISA on the day on which they were fed; 80% were positive 12 days later when the test was concluded. Feeding on alternate prey decreased reactivity slightly. Fifty percent of the spiders initially fed a single first instar DFTM and subsequently fed 3 non-DFTM larvae tested positive after being held 12 days at a constant 24°C.

Field trials of this assay were conducted in 1979, 1980 and 1981 with arthropods collected in association with a native population of DFTM. Samples were taken at budburst from white fir from El Dorado National Forest, California. No cross-reactions with alternate prey were found in 1979 or 1980; phytophagous mirids did show false positive reactions in 1981. Cross-absorption of the conjugate prior to ELISA eliminated these heterologous reactions.

All but rare species of arboreal arthropod predators were confirmed as having consumed first or second instar DFTM. A fortuitous 800-fold decline in the DFTM population occurred over these 3 study years. The proportion of predators of all guilds declined non-linearly with this decline in DFTM density. Spiders in each guild (diurnal active hunters, nocturnal active hunters, ambush hunters, larger web-spinners and minute web-spinners) consumed DFTM in linear proportion to the relative abundance of DFTM as one potential prey, i.e. were non-selective predators. Predaceous hemipterans, principally mirids, showed a strongly logarithmic response in the same analysis.

Arboreal Arthropod Predation on Early Instar Douglas-fir Tussock Moth

by

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Although my contact with Don Dahlsten has been brief, he allowed me to accompany his field crew and shared his field data when no gain was evident for him. I also wish to thank Mauro Martignoni for unfailing supplies of tussock moth larvae and Dick Mason and his associates for their support of this work.

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PREFACE

Chapters II, III and IV which deal with the technical development and laboratory exploration of ELISA were coauthored with W. P. Stephen (Fichter and Stephen 1979, 1981). Dr. A. R. Moldenke identified all field-collected specimens cited in Chapters V and VI. Dr. D. L. Dahlsten also coauthored the paper presented as Chapter V; his sites, procedure and field crew were used for all sampling in the Sierra Nevada, California.

This work was initially supported by a National Science Foundation ecology grant; a Crag minority grant and a cooperative agreement through the US Forest Service provided funds for the field analysis.

ARBOREAL ARTHROPOD PREDATION ON EARLY INSTAR DOUGLAS-FIR TUSSOCK MOTH

I. INTRODUCTION

In recent years several major studies of arboreal lepidopteran defoliators have been conducted. These studies, each of which focused exclusively on one herbivore, provide what little is known of the arboreal arthropod community. Investigations have been made of winter moth, Operophtera brumata (L.) (Varley et al. 1973); gypsy moth, Porthetria dispar (L.) (Campbell 1976); eastern spruce budworm, Choristoneura fumiferana (Clem.) (Morris 1963); and Douglas-fir tussock moth, Orgyia pseudotsugata (McDunnough) (Mason 1976, 1981; Dahlsten et al. 1977; Mason et al. 1983; Mason and Overton 1983). In each of these studies larval mortality was identified as a major factor in maintaining the species at endemic levels; arthropod predation was implicated as a key regulating component. However, both qualitative and quantitative data on the differential roles of various polyphagous arthropod predators on the target-prey are lacking in all studies.

The key factor causing population density changes in winter moth, as well as the primary cause of death in this species, was identified as "winter disappearance" (Varley et al. 1973). This term included all mortality between estimated number of fertile eggs laid and numbers of surviving large instar larvae. No estimate of the contribution of arthropod predation, as one factor in the multitude of biotic and abiotic causes of death, was attempted.

The loss of gypsy moth larvae, especially instars I - III, is one of the major factors in the dynamics of both sparse and dense populations (Campbell 1976). This mortality was broadly attributed to dispersion and "other". As with winter moth, no investigation of the role of

invertebrate predators was pursued.

In detailed studies centered on outbreak populations of the eastern spruce budworm, Morris (1963) concluded that if predation is important to the dynamics of budworm populations, the pressure must occur at endemic levels or during early years of population release. Spiders were considered important contributors to total predation both because of their abundance (8 spiders were found for every insect predator over an 8 year period) and because of their extended activity period each season.

Loss of early instar (I - III) Douglas-fir tussock moth larvae (DFTM) was found to constitute 66 - 92% of intra-generation mortality and to be a key factor in inter-generation population change (Mason 1976, 1981; Dahlsten et al. 1977; Mason et al. 1983; Mason and Overton 1983). This population decline was attributed to dispersion and arthropod predation, two factors Mason judged more important to an endemic than an outbreak population.

Polyphagous arthropod predators abound in the forest canopy. Predation by invertebrates upon other invertebrates has been extremely difficult to document and nearly impossible to quantify. Direct documentation depends upon observation which is time-consuming, site-specific, expensive, heavily biased and difficult to interpret. Serological techniques can provide indirect evidence of predation while avoiding most of the limitations of direct observation. To be useful, however, a technique must be sensitive enough to identify the consumption of one first instar larva and rapid enough to permit the analysis of many hundreds of specimens to minimize sampling error.

In 1979, through a Crag minority grant from the U.S. Forest Service, a pilot study was initiated to determine if it was possible and practical to detect and quantify

predation by arthropods on DFTM in the coniferous canopy using the highly specific and sensitive immunotechnique, ELISA. In this test the specificity of antibody recognition of one antigen is used to identify the prey, while the amplification resulting from conversion of many molecules of substrate by one molecule of enzyme is used to gain sensitivity. An antibody reactive only with DFTM proteins was first developed using DFTM hemolymph to elicit antibody production because this tissue would be consumed by all arthropod predators (Chapter II). Feeding trials were then established to determine the degredation rate of DFTM antigens in hemipteran and spider predators (Chapters III and IV respectively).

The culmination of this work involved testing field-collected specimens. Through the cooperation of D.L. Dahlsten, University of California, we collected potential arthropod predators and prey during early season samples from white fir in the El Dorado National Forest, California, in 1979, 1980 and 1981. Thousands of predators were individually tested with ELISA to determine which contained tussock moth antigens. The community from which these specimens were taken is described in Chapter V and the ELISA results are presented in Chapter VI.

No serological technique can distinguish predation from saprophagy or identify secondary predation (one predator feeding on another predator which has previously eaten, but not fully digested, the target prey). ELISA cannot tell us precisely how many of the target prey were eaten by individual predators and thus cannot quantify mortality of the prey. However, ELISA may be employed to identify which prey species are being consumed in communities where quantification by direct observation is impossible and can be used to generate estimates of the relative consumption of different prey species by different species of predators.

For a multi-prey, multi-predator community such as the canopy, data detailing the acceptability of each prey species to each predaceous species must be available prior to manipulations to extend and quantify these models. Only then can the many assumptions which underlie generalizations on predator-prey dynamics be tested.

Objectives of this work:

1) To develop a serological assay which would distinguish predation on DFTM from predation on coexisting lepidopteran larvae and would have the sensitivity to detect predation upon a single early instar DFTM.
    A) To produce antibodies reactive with a DFTM tissue ingested by every arthropod predator.
    B) To reduce or eliminate heterologous reactions of this antiserum with coexisting lepidopteran larvae from Mares Egg, Oregon.
    C) To develop an ELISA, using these antibodies, with the sensitivity to monitor feeding on a single first instar DFTM.
2) To investigate the time-dependent loss of antigenic response by DFTM antigens homologous to these antibodies in a representative hemipteran predator, Podisus maculiventris, and in representative fir-inhabiting spiders.
3) To employ the assay to identify the arthropod predators of a native, endemic and unmanipulated DFTM population immediately after the budburst-timed dispersal of the larvae.

II. SELECTION AND USE OF HOST-SPECIFIC ANTIGENS

ABSTRACT

Consumption of a single species of prey by a polyphagous arthropod predator may be identified serologically if antigens unique to that one possible prey can be located. These specific antigens can be used to elicit antibodies which will then not cross-react with any coexisting prey in the defined universe. This paper present a method for 2-dimensional immunoelectrophoretic separation and characterization of prey antigens to allow location of specific fractions. Antibodies formed against these specific antigens can then be used in enzyme-linked immunosorbent assay (ELISA), a rapid and extremely sensitive test which will allow the screening of hundreds of potential predators for consumption of the prey species.

INTRODUCTION

Attempts to validate models of insect community structure have emphasized the lack of definitive data on the role of arthropod predation within these systems. Currently the overall impact of predation is assessed essentially by measuring total mortality, subtracting mortality due to factors other than predation (e.g. abiotic influences, parasitism), and attributing any residual mortality to predation. Even scantier information is available on the significance of any single species of polyphagous predator to the regulation of a prey population.

None of the many approaches to the latter problem has proven capable of accurately indicating the consumption of a single small prey by a polyphagous predator (Boreham and

Ohiagu 1978). Whereas a serological reaction appears potentially capable of yielding such information, the precipitation methods currently employed lack sensitivity and objectivity and are demanding of both time and materials.

Microtechniques developed in the past 10 years hold promise of overcoming these limitations. Two-dimensional immunoelectrophoresis provides a rapid means of selecting for an antigen(s) unique to any one of the coexisting species in a defined ecosystem. The chemical capability of the antibody prepared against the host-specific antigens must then be exploited in a test sufficiently sensitive to detect the micro quantities of prey tissue in a predator after a single feeding. Further, its applicability to ecosystem study demands that such a test be simple and inexpensive so that hundreds of predators may be analyzed. Enzyme-linked immunosorbent assay is ideally adapted to such analysis (Scharpe et al. 1976, Voller et al. 1977, Wisdom 1976).

In ELISA, an enzyme is covalently linked to the previously selected host-specific antibody. When this antibody subsequently reacts with host-specific antigen, the bound enzyme is carried along. The ELISA test is based on the message amplification inherent in enzyme action: a single molecule of enzyme converts many molecules of substrate. Whereas the binding of a few molecules of antigen with a few molecules of antibody usually cannot be directly detected without amplificaion, the rate of substrate conversion by a few molecules of enzyme can be accurately and objectively measured.

The ELISA double antibody sandwich technique was used in this study. This procedure utilizes the antibody as a glue to immobilize antigens on a microtitration plate. Once the antigens are firmly attached, the enzyme coupled to host-specific antibody is added. This forms a 3-tiered

"sandwich" of antibody-antigen-(antibody-enzyme) to which the enzyme substrate can be added. By choosing an enzyme which converts a colorless substrate to a colored product, the positive reaction can be estimated visually or read spectrophotometrically.

There have been recurrent outbreaks of DFTM in the western United States and clarification of the role of individual species of arthropod predators as population regulators in the intervening years would be valuable. The initial objective of the program was to isolate a DFTM-specific antigen, and in the ensuing discussion this is referred to as "host-specific". It must be emphasized that such DFTM antigen need not be unique among all possible lepidopteran antigens, but rather it is unique only among those coexisting, contemporaneous lepidopterous larvae in the ecosystem being examined.

MATERIALS AND METHODS

DFTM Populations: Three geographically distinct DFTM populations were available as antigen sources at different times during this study. The initial DFTM was a Riverside, California, F_6 population given to us by R. Beckwith of the Forestry Sciences Laboratory, Corvallis, OR. The second population came from Trigo Canyon, NM, collected as F_1 eggs. The third DFTM used as an antigen source were supplied by M. Martignoni of the Forestry Sciences Laboratory, Corvallis, OR, from Goose Lake, CA, F_5 larvae (reference number 207/208).

Field collected eggs were surface sterilized with two 15 min washes in 2% sodium hypochlorite. All 3 populations were laboratory reared at 26°C in plastic petri dishes on tussock moth medium #65 (Forestry Sciences Laboratory, Corvallis, OR).

Initial Preparation of DFTM Antigens: Hemolymph was drawn from a dorsal incision in individual larvae previously starved for at least 18 h. It was stored undiluted at 4°C with no melanization inhibitors. A precipitate formed during storage at 4°C; only the fluid portion was used.

Preparation of Possible Cross-reacting Antigens: A collection of other lepidopteran larvae coexisting with second stage DFTM in the Fort Klamath region of Oregon was made in the spring of 1977. These approximately 15 species, principally Geometridae and Tortricidae, varied greatly in biomass. Due to this size variation, withdrawal of blood was not feasible. Instead a whole-body squash was made into a roughly equal volume of a 0.1M phosphate buffer (pH 7.2) and spun for 5 min at 10,000 RCF to compact the solid matter. The decanted fluid was stored at 4°C; this fluid also formed a precipitate with time but again only the fluid portion was used.

Preparation of Non-specific Antisera: Initial injections of antigen from Riverside DFTM into 2 one-year old rabbits were made using 0.5 ml of antigen emulsified in an equal volume of Freund's complete adjuvant. Nine months later each rabbit was injected subcutaneously with 1.0 ml from Trigo Canyon DFTM. The adjuvant was Arlat 16 in a 5 parts antigen to 7 parts Arlat 16 ratio. The material injected was later found not to have formed an emulsion. Therefore one week later each rabbit was again injected subcutaneously with 1.0 ml of Trigo Canyon DFTM antigens emulsified in a 5:7 ratio of antigen to Freund's incomplete adjuvant. For each of these subcutaneous injections the number of lymph nodes activated was optimized by dividing the 1.0 ml of material injected among 8 flank sites.

Assessment of Antisera: One month after the third injection approximately 30 ml of blood were removed via cardiac puncture. The serum was separated and stored at 4°C with merthiolate in a 1:10,000 concentration.

Electrophoresis in polyacrylamide - The polyacrylamide disc electrophoresis was effected with a 7% separating gel cross-linked with bis acrylamide and overlayed with a stacking gel (Davis 1964). Tubes 8 cm long were run in a Canalco 1200 apparatus at 4°C with a voltage gradient from 10 to 20 V/cm. (All voltage gradients cited are average gradients measured terminal to terminal.) The gels were very briefly stained with Coomassie Brilliant Blue R and then rinsed in water; no destaining was necessary. The protein bands became visible over the next 24 to 48 h.

Electrophoresis in agarose - A 1% agarose (Sigma type 1, low EEO) in barbital buffer formed as a 1 mm thick, 8x9 cm slab was run horizontally in a BioRad cell #1400 in which gel cooling to 4°C was provided by direct contact with a water-cooled platen. The slab could be rotated 90° for a 2-dimensional run. Voltage gradients from 5 to 20 V/cm were employed. Two barbital buffers were used: 0.014 M sodium diethyl barbiturate containing 0.014% sodium azide (BioRad Immunoelectrophoresis Buffer I diluted 1:6 over package instructions), and 0.075 M sodium diethyl barbiturate containing 0.04% calcium lactate (BioRad Immunoelectrophoresis Buffer III, Laurell).

The selection of antigens specific for DFTM is diagrammed in Fig. II.1. A layered slab of agarose was prepared using 1% agarose in barbital poured to a depth of 1 cm. The agarose bed was overlaid to a depth of 1 cm with 1% agarose in barbital containing 0.25 ml of the pooled extract from the other coexisting lepidopteran larvae (Fig. II.1B). This 2 cm of gel was then overlayered with agarose in barbital containing roughly 0.8 ml of the non-specific antiserum. A parallel run was made using 2 cm of plain

agarose into which the pooled extract was not incorporated.

In order to locate DFTM-specific antigens it was necessary to gel-fractionate the whole hemolymph sample and then pass it electrophoretically through the superimposed gel containing the pooled sample into the agarose containing non-specific DFTM antiserum. A 5 µl sample of whole DFTM hemolymph was electrophorated on polyacrylamide because of its fractionating superiority over agarose. The gel was then sliced longitudinally and laid along the agarose block behind that into which the pooled larval extract had been incorporated (Fig. II.1D).

Electrophoresis was conducted at 5 V/cm for 24 h and upon staining revealed a pattern similar to that shown in Fig. II.1E. By aligning a separately stained disc polyacrylamide gel run in parallel with the half gel used on the agarose, the protein bands which corresponded to the precipitin peaks were identified as fractions unique to the DFTM larvae.

Production of a Specific Antiserum: Once the DFTM-specific area of a gel was located, 12 polyacrylamide disc gels overlayed with 15 µl DFTM hemolymph were run in parallel. Of these one was stained to identify the desired band area. This portion of the other 11 unstained gels was removed, homogenized with a small volume of sterile distilled water, and injected subcutaneously at 8 sites into the rabbit whose antiserum was used to identify the specific peaks referred to above. One month was allowed for the development of optimum ELISA antibodies before 30 ml of blood was removed via cardiac puncture and the serum stored as above.

Precipitation of Immunoglobulins: Ammonium sulfate was added to the serum to precipitate the immunoglobulin fraction; the ratio of fluids was 1 part serum: 9 parts

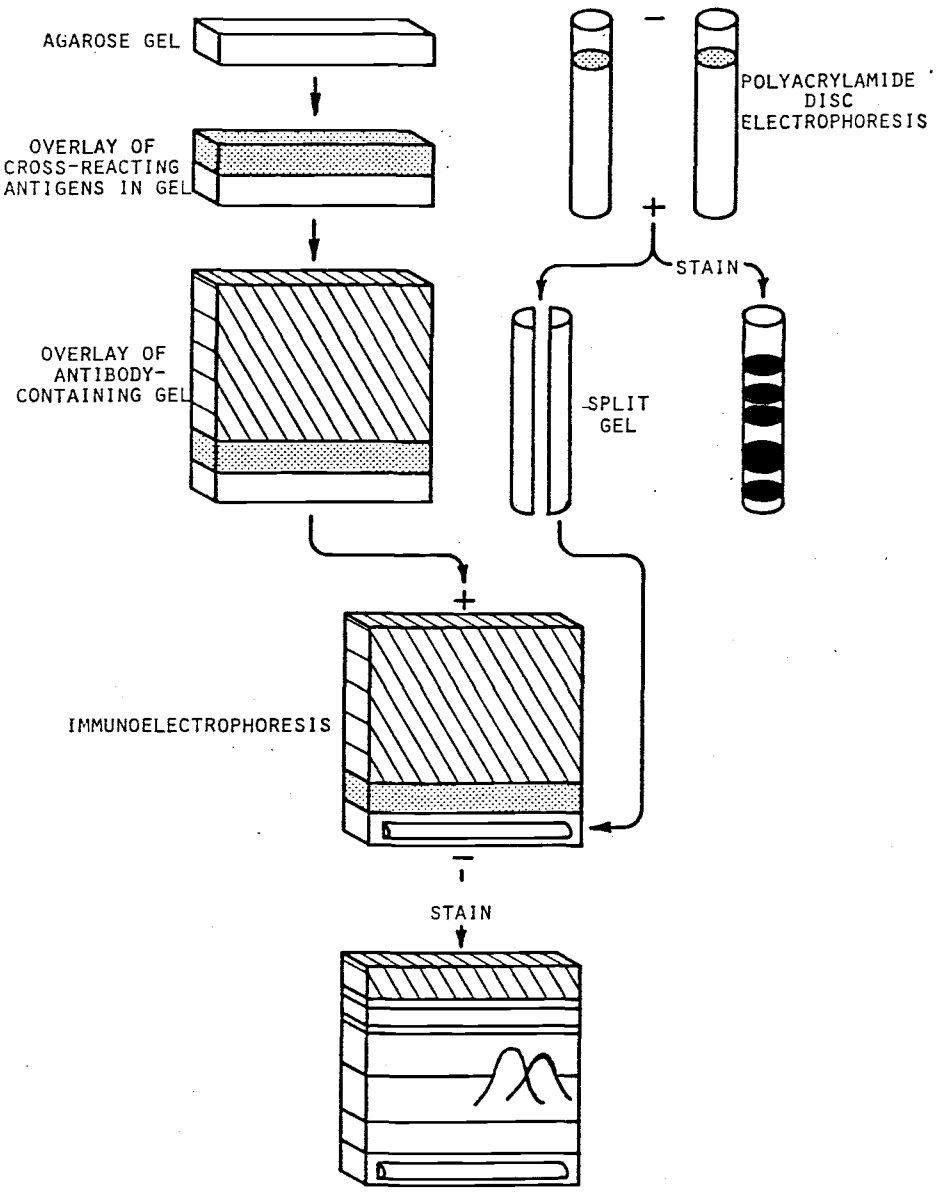


Fig. II.1 Diagrammatic representation of steps in the selection of specific antigen.

distilled water: 10 parts saturated ammonium sulfate. The precipitate was pelleted then resuspended in 2 ml of 0.01 M pH 7.2 phosphate buffered saline (PBS) and dialyzed 3 times against the same PBS. The OD_{280} of this fluid was adjusted to ca. 1.4 (about 1 mg/ml). Storage was in siliconized glassware at -20°C.

Conjugation of the Immunoglobulins with Horseradish Peroxidase: A one-step conjugation of horseradish peroxidase (Sigma type VI, RZ 3.0) with the immunoglobulins was done by dissolving 5 mg horseradish peroxidase in 2 ml of immunoglobulin solution and adding fresh gluteraldehyde solution to a final concentration of 0.06%. This mixture was allowed to stand 5 h at room temperature before dialyzing 3 times against 0.01 M PBS at 40°C. Bovine serum albumin was then added to about 5 mg/ml, and the solution stored at 40TC.

ELISA: Microtitration plates (Cooke #220-29) were coated with DFTM-specific antiserum by filling each well with 250 µl of 1 µg/ml immunoglobulins diluted in 0.06 M pH 9.6 carbonate buffer and allowing them to stand 5 h at 30°C in a high humidity chamber. These plates were stored at 4°C for a maximum of 1 week before being used.

The test was initiated by shaking out the antibody solution and washing each well thoroughly with PBS containing 0.05% Tween 20 (PBS/T).

A 200 µl volume of antigen, diluted as desired in PBS/T, was added and allowed 30 min at 30°C to complex with the antibody. This fluid was then shaken off and a multiple wash with PBS/T was done as before.

A 200 1 volume of immunoglobulin conjugated with horseradish peroxidase and diluted 400^{-1} with PBS/T was next allowed 30 min at 30°C to conjugate with the adhering antigen before being shaken off. After the wells

were washed as before, 200 µl of substrate was added. This substrate was distilled water containing 0.003% H_2O_2, 1% methanol, and 0.01% O-phenylene diamine. After development for 30 min at 30°C in a dark chamber, 25 µl 8 N H_2SO_4 was added to each well. The amount of color was then either visually estimated or read in a 1 cm flow-through cell at 490 nm. This technique is a combination of the methods of Clark and Adams (1977) and Walls (1977).

RESULTS

Selection for DFTM specific antigens was made on the basis of precipitates resulting from the methods illustrated in Fig. II.1. An arbitrary ratio of 50 volumes of pooled extract to 1 volume of DFTM hemolymph was utilized to approximate the protein levels in both samples.

The electrophoretic separation of the DFTM hemolymph on polyacrylamide rather than agarose was necessary to effectively separate its antigenic components in the first dimensional run on the gel. Laying the half gel on the basal agarose block in the system and electrophorating the agarose unit resulted in the formation of 5 uniform precipitin lines across the upper block plus 2 precipitin peaks. The antigens present in the pooled sample appeared as 5 precipitin lines parallel to the interfaces of the gel blocks because of their uniform distribution in the intervening agarose block (Fig. II.1D). Only host-specific antigens were present as precipitin peaks.

The relationship between the amount of antigen and the absorbance of the enzyme product following ELISA is shown in Fig. II.2. The enzyme product from the pooled lepidopteran sample was detectable at dilutions up to 128. This suggests that further DFTM antigen purification is necessary if absolute antigen specificity is sought.

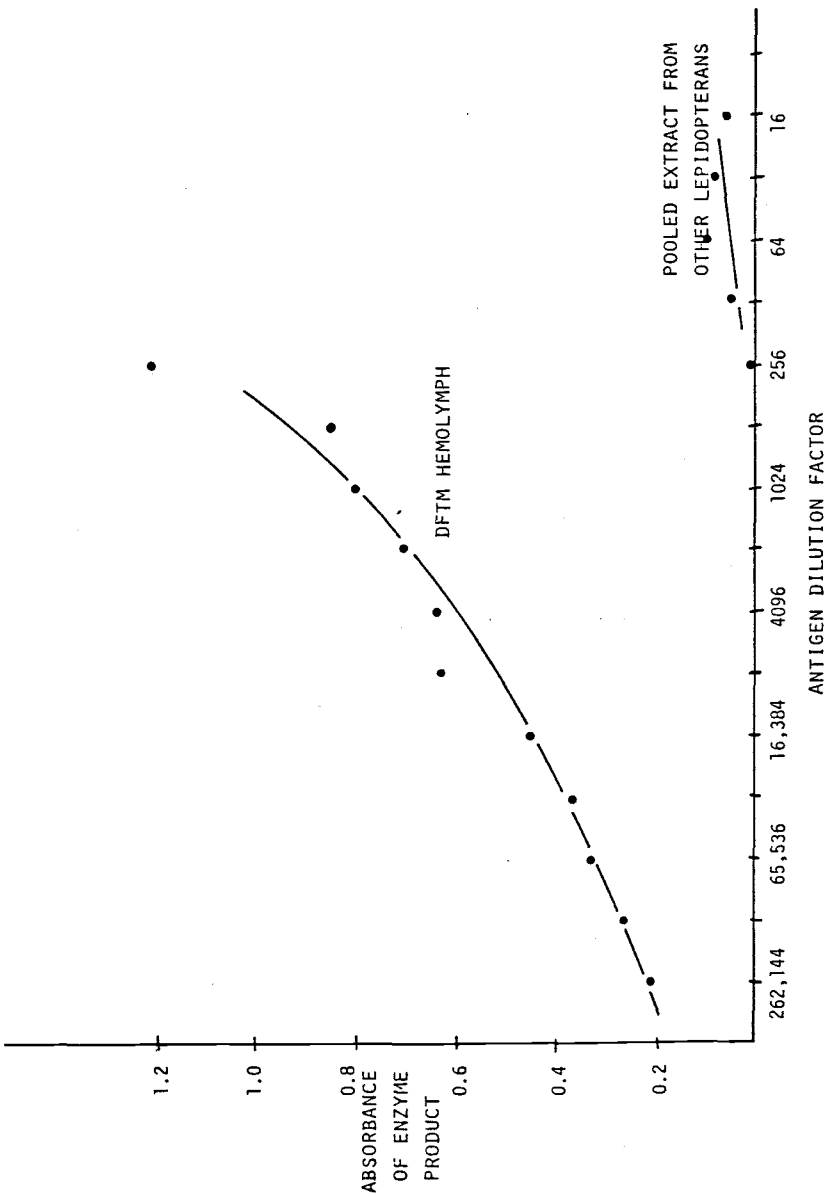


Fig. II.2 Absorbance of the enzyme product as a function of antigen dilution.

However, the absence of overlap in absorbancies between DFTM and pooled larvae indicates such purification, although desirable, may not be necessary. The detectability of DFTM antigens at dilutions of up to 262,000 attests to the sensitivity of ELISA. Controls consisting of a squash of house flies (Musca domestica Linnaeus), of Indian meal moth larvae (Plodia interpunctella), and of variegated cutworm larvae (Peridroma saucia Hubner) were also tested at dilutions between 1:8 and 1:128. The absorbance was zero for all controls at all dilutions.

DISCUSSION

The predators of DFTM with which this study was concerned were all blood-sucking arthropods; thus DFTM hemolymph was the tissue selected as the antigen source. Although withdrawal of blood was more time consuming than simply squashing the entire insect, it offered one great advantage in these preliminary studies: no extraneous antigens from hairs or other unconsumed body structures had to be considered. Not only would these further complicate extraction procedures, but the possibility of selecting a compound not available to blood-sucking predators was eliminated.

The cross-reaction between antibodies prepared against one species with antigens of related species has been widely noted in precipitin tests. It was thus essential to screen the tissue from all other species of lepidopterous larvae coexisting with the DFTM in the system being studied to insure no cross-reaction with DFTM-specific antibodies. As neither the species identity of the coinhabitants nor the extent of cross-reaction was of concern, a pooled sample of all other contemporaneous Lepidoptera was adequate for the test.

Antisera prepared against mixed antigen sources of unknown composition are of limited utilitarian value in all precipitin techniques. An optimal ratio between an antigen and its homologous antibody is usually necessary to achieve maximal precipitate formation. When dealing with a potpourri of antigens, such as that found in whole hemolymph, there is a high probability that the optimal antigen-antibody ratio will differ for each pair of homologues. Thus any cross-reaction between the antigen-heterogeneous hemolymph and its derived antiserum will result in the precipitation of but a few of those homologous pairs which happen to be represented in appropriate ratios.

ELISA does not rely on precipitation. Attachment to the polystyrene surface allows the detection of any complexing among homologous pairs via the attached enzyme. Multiple antigenic sites on an antibody, a requirement for precipitate formation, are not necessary for this one-on-one reaction. In this way ELISA greatly increases the number of usable antigens and amplifies their detectability at high dilution levels.

CONCLUSIONS

The sensitivity and mass sampling advantages of ELISA could prove of enormous benefit in a field such as arthropod predator-prey relationships. The ability to select any antigen from an insect and produce a specific antiserum to it may extend its applicability to other physiological problems.

The adaptation of a relatively new technique to a new application is obviously both complicated and time consuming. With ELISA, however, the potential benefits of this system seem worth the work.

III. TIME RELATED DECAY IN PREY ANTIGENS INGESTED BY THE PREDATOR Podisus maculiventris (HEMIPTERA, PENTATOMIDAE) AS DETECTED BY ELISA

ABSTRACT

Individual nymphs of the predaceous pentatomid Podisus maculiventris Say were each fed a single first instar DFTM and held without further feeding at constant temperature for a known number of days before being frozen. ELISA was used to examine these predators for the presence of prey antigens. The concentration of prey antigens in these predators declined at a linear rate over the 7 days they were held post-feeding. Detectable antigens remained in 50% of the predators after three days at 24°C. On the day in which the prey was consumed (day 0) only 80% of the unstarved predators had detectable prey antigens which suggests the possibility of instinctive killing of prey with little or no subsequent ingestion. The amount of prey antigen in molted and unmolted predators was not statistically distinguishable; although molting interrupts feeding, digestion of the antigen(s) employed in this study seems to be continuous.

INTRODUCTION

Assessment of predation in a natural habitat by polyphagous arthropods has frequently been based on exclusion techniques or on extrapolation from laboratory feeding trials because more objective means of evaluating such predation has not heretofore existed (Boreham 1978). This is evidenced by the general lack of definitive insect predation data in models of insect community structure and, aside from empirical evidence on prey regulation as a result of parasite introductions, the paucity of data on

the role of any single polyphagous predator species in the regulation of a target-prey species. Acquisition of such data is contingent on development of an indirect method of ascertaining the feeding behavior of an individual predator. Serological techniques appear most promising (Miller 1979) but only if a method can be perfected with sufficient sensitivity to detect antigen(s) of a single early instar target-prey species from among the multitude of antigens of other prey species coexisting with a polyphagous predator.

A recent report on the use of ELISA in predator-prey studies suggests that such levels of antigen detection are possible. The method is objective, easily used for hundreds of individual tests, and sufficiently sensitive to detect prey antigens in hemolymph dilutions of more than 260,000. Its objectivity is based on the preparation of an antiserum which will react uniquely with an antigen(s) from the target-prey species. Its sensitivity is a function of the linkage of an enzyme to this unique target species antibody(ies). The presence of this enzyme is then detected and amplified by the conversion of enzyme substrate for spectrophotometric quantification.

The parameters which limit field applicability of ELISA remain to be established. Of immediate concern is the effect of digestive processes of predators on target antigen(s), i.e. the period following prey ingestion during which prey antigen can be detected. It is anticipated that the length of this period may be influenced by the developmental stage of both the prey and predator, the effect of temperature on rates of digestion and absorption, the effect of ingestion of alternate prey before and/or after that of the target species, and the effect of differences in digestive process between arachnids (intracellular) and insects (extracellular).

In this study laboratory feeding trials were conducted

to determine the time-decay rate of the target antigen(s) of DFTM following ingestion by the pentatomid predator, Podisus maculiventris.

MATERIALS AND METHODS

The predator, obtained from W.H. Whitcomb, Gainesville, FL, was kept in continuous culture at 24°C, 16L:8D, 60% R.H. in 15x100 mm plastic petri dishes. The number of Podisus per dish varied between 15 (early instars) and 5 (adults). Celery and Tenebrio spp. larvae of appropriate size were made available three times per week. No abnormalities in final body size, rate of development, or fecundity (Richman and Whitcomb 1978) were noted under this rearing regime.

Surface sterilized DFTM eggs were obtained from M. Martignoni from his F_{16} Goose Lake stock (reference # GL-1). Larvae were hatched and reared in 15x100 mm plastic petri dishes using diet # 65 (Forestry Sciences Laboratory, Corvallis, OR) in physical conditions identical to those for Podisus.

Feeding trials were conducted by placing one unstarved second instar through adult stage Podisus together with one first, second, or third stage DFTM into a 50x9 mm sealable plastic petri dish. Contact was allowed for up to 8 h at one time under the same physical conditions used for rearing. The Podisus which fed during one trial were given celery and maintained individually in a 50x9 mm plastic petri dish for from 0 to 7 days before being frozen at -20°C. Those which did not feed were given access to celery overnight; the following day the celery was removed and a DFTM was again offered as food. The DFTM were kept in a 100z15 mm petri dish with access to their diet when not with Podisus.

Predator molting in the interval between feeding and

freezing was noted; those Podisus (9 of 225) which died or appeared moribund were excluded from the analysis.

Each fed Podisus , kept frozen for approximately 180 days, was tested in duplicate via ELISA against antiserum previously developed against DFTM. Individual specimens were squashed in 0.45 ml phosphate buffered saline (pH 7.2), permitted to stand for 24 h at 4°C, and the fluid portion used for ELISA. The procedure of Chapter 2 was followed with two modifications: no Tween 20 was used in any phase of the test and 0.05% bovine serum albumin was used in the conjugate solution. Controls on each titration plate consisted of Podisus fed only on Tenebrio, Tenebrio body fluids, and a dilution series of DFTM hemolymph based on powers of 2. A total of ten plates (96 wells each) were used, two on each of five days on which analyses were conducted. Substrate conversion was read spectrophotometrically.

Statistical Analysis: The resulting data were interpreted using two different statistical analyses. In one a threshold value was calculated each day, that threshold being the mean of the conrol wells plus two standard deviations (ca. 95% one-sided confidence limit). The spectrophotometric reading from the trial-fed Podisus were then evaluated as falling above the threshold value or below. A spectrophotometric reading greater than the threshold value represented a positive reaction (i.e. the presence of DFTM antigens) for 95% of these values. Conversely, a reading below the threshold signified the absence of DFTM antigens.

The second analysis was concerned with combining the actual spectrophotometric readings. Day to day variability in reagent concentrations and incubation time introduced a correctable error into each trial-fed Podisus value, the correction being based on the dilution series included on

each plate. Since the dilutions and time had been chosen to produce to near-linear portion of the enzyme kinetic curve, any small change in these values would be reflected in a change in the slope and intercept of the regression line. The linear regression line from each of the five days on which ELISA plates were tested needed to be transformed to coincide with a single arbitrary reference line. The transformations resulting from unifying the dilution references lines were then used to adjust the trial-fed Podisus absorbance values, a different transformation being used for the data from each day. This adjustment effectively minimized day to day variability to allow a direct comparison of the absorbance values of each Podisus. This is how it was done:

The reading of the control wells lacking DFTM antigens were regarded as background variation. The mean of the control wells for each day was therefore subtracted from the readings for all other wells prior to any other calculations.

Following the regression of the dilution series on the spectrophotometric reading, various transformations were tried with the single aim of improving linearity (i.e. maximizing r^2). These individual regression lines were then compared to a single arbitrary reference line which served to unify all the regression lines from all the days. The individual regression lines fit the form:

$$y = mx + b$$

where y is the original absorbance reading minus the control value for that day and x is the dilution. The reference line can then be written as:

$$y' = m'x + b'$$

where y' is the adjusted absorbance value (i.e. the value adjusted for day to day variations). Then the equation:

$$y' = m' (y - b)/m + b'$$

can be used to adjust the value y to y'.

Thus day to day variation in each set of data was minimized by treating the mean of the duplicate spectrophotometric readings from each trial-fed Podisus as follows: 1) subtracting the mean of the control wells from the day on which the Podisus was tested to obtain the value of y; 2) determining the values for m and b from the dilution series run the day the Podisus was tested; and 3) combining the values of y, m and b thus obtained with m' and b' from the arbitrary reference line in the relationship described above. The y' values thus calculated were considered directly comparable.

Best linearity for the regression between the absorbance value and the dilution was achieved by regressing the mean of the duplicate absorbance readings against the reciprocal of the $\log_2$ of the dilution factor (the $\log_2$ of the dilution factor is an integer). Correlation coefficients for the individual plates were between 0.71 and 0.96.

Multiple regression of the reciprocal of the $\log_2$ of the dilution factor against the mean spectrophotometric value corresponding to that dilution and the day and plate on which each series was run indicated that r^2 was increased by 2% when the day was included in the model. No increase in r^2 to 4 significant figures resulted from including the plate. Calculations were therefore based on formation of a single regression line from the two plates run each day, plate-to-plate variation being excluded from any further consideration.

RESULTS AND DISCUSSION

The number of individuals of various stages of Podisus noted to have consumed an early instar DFTM are recorded in Table III.1. These data are not meant to imply that an equal number of trials or a comparable contact time was provided to every possible combination of instars of predator and prey, i.e. if a second instar Podisus refused second instar DFTM, the larger third instar of the prey was not offered. Rather our goal was the assessment of the effect of ingestion and digestion on specific prey antigens. Predation was fostered through pairing developmental stages of predator and prey which maximized acceptance of the latter.

The relationship between the number of days a Podisus was held after feeding and the percent of those which reacted positively to DFTM antiserum in ELISA is shown in Fig. III.1a. In the course of the feeding trials each Podisus was kept in association with a prey until a dead, apparently fed upon larva was found in the dish. Thus it was surprising that of those Podisus frozen the day on which prey killing occurred (day 0), only 80% responded positively.

The 20% negatively responding predators may reflect a condition in which prior satiation may result in innate killing of prey without subsequent ingestion. The use of unstarved predators was expected to result in a lower mean spectrophotometric reading and a much higher variance due to a smaller and more variable portion of the prey actually being ingested (Fig. III.1a, b). This is believed to more nearly reflect the nutritional state of field collected predators.

The curve resulting from a regression of the number of days a Podisus was held following feeding and the mean spectrophotometric reading for each day is plotted in Fig.

Table III.1 Number of each instar of DFTM larvae consumed by respective stage of Podisus.

		DFTM stage consumed		
		1	2	3
Podisus stage consuming	2	31[a]	1	0
	3	25	2	0
	4	19	16	1
	5	6	58	40
	A	0	2	24

[a] The number of individuals consumed.

Table III.2 A comparison of absorbance values and mean larval mass relative to the DFTM stage consumed in extracts from matched Podisus. Data are presented from Podisus matched for stage and number of days held post-feeding.

		Mean % positive[b]	Mean OD - n[c]	Mean larval mass (g) - n[c]
DFTM stage	1	25.0	0.007 - 10	0.006 - 40
	2	33.2	0.026 - 49	0.035 - 54
	3	57.8	0.059 - 28	0.058 - 92

[b] The mean of 7 daily means.

[c] Number of samples.

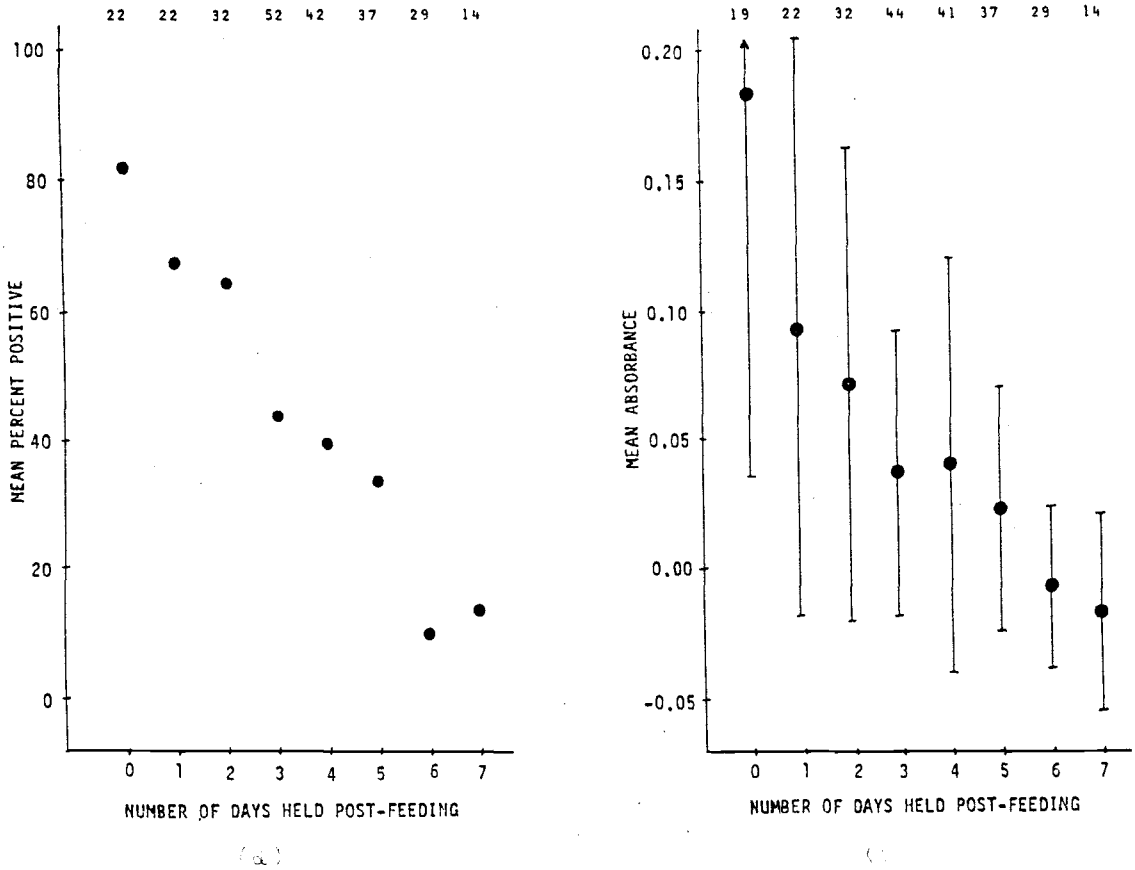


Fig. III.1 Degradation of DFTM antigens at 24°C in the predator Podisus maculiventris. The numbers along the top indicate the number of individuals tested.

(a) Percent of Podisus responding positively relative to the number of days held post-feeding. Data based on all predator and prey instars tested.

(b) Mean absorbance + SD relative to the number of days each Podisus was held post-feeding. All absorbance values were transformed to a single reference dilution curve as described in the section "statistical analysis".

III.1b. The difference between the number of samples incorporated into Fig. III.1a and b results from omitting from III.1b one plate which displayed extremes in adjusted absorbance readings.

Some error is present in the very highest absorbance readings because these values lay outside the control curve limits. The upward displacement of the value for day 0 (Fig. III.1b) may be due to this error in extreme values.

The linear decline in the percent of Podisus reacting positively over 7 days (Fig. III.1a), and in the mean absorbance value over the same 7 days (Fig. III.1b), suggests a constant rate of digestion at a constant 24°C. Since ELISA actually measures changes in concentration of but a few selected antigenic components of DFTM, additional studies are necessary to determine if a constant rate of digestion is generally true of this species, and to what extent the rate of digestion changes with different chemical components of the meal.

The percent positive responses of fed Podisus dropped to 45% by day 3 and to 15% of those analyzed after a week's starvation (Fig. III.1a). Although the variance of the absorbance reading was less than that of samples run on day 0 and day 1, it remained relatively high throughout the test period. This further supports the contention that the amount of prey antigen ingested at the time of each kill reflects the level of predator satiation prior to ingestion of the DFTM. Nevertheless it is abundantly clear that, in this system at least, ingested antigen(s) can be detected in 15% of the predators for a week or longer after feeding; the capacity to detect the target prey antigen(s) is apparently a function of the amount ingested by the predator at the time of feeding. The data suggest that not only does prey killing without body fluid ingestion occur, but that the quantity of prey body fluids ingested by sated Podisus may form a continuum of from zero (20%) to complete

(15% positive on day 7). Transposing these observatons to field conditions does not necessarily imply that an automatic 20% error (underestimate) in target prey species kill is to be expected in daily samples of predaceous hemipterans. Rather, the slow digestion rate (50% positive responses after 3 days starvation) should compensate for those predator-prey bouts in which ingestion does not occur due to predator satiation.

Those Podisus which molted following feeding and prior to freezing were compared with those which did not by matching: Podisus instar, DFTM instar consumed, and the number of days held following feeding. Of the 27 molted Podisus , 37% reacted positively; 39.1% of the 69 matched unmolted Podisus were positive. Molted Podisus had mean spectrophotometric readings of 0.046 (n = 26. s = 0.066), whereas unmolted had a mean of 0.040 (n = 54, s = 0.067). This close agreement between molted and matched unmolted Podisus in both the percent positive and mean spectrophotometric values indicated that little or no disruption in digestion occurs during ecdysis even though feeding itself is interrupted.

A comparison of the absorbance values from Podisus extracts relative to the DFTM instar consumed reflects the obvious: the larger the prey, the higher the ELISA absorbance value (Table III.2). To avoid predator size and starvation time biases, these comparisons were based only on fourth and fifth instar Podisus each of which had consumed a single first through third instar DFTM and each of which had been starved for the same number of days prior to freezing.

CONCLUSIONS

The variability in the ELISA absorbance values of the trial fed Podisus was great; even under controlled

laboratory conditions large numbers of predators were required for testing. Error analysis ascribed the bulk of the variability to the organism(s) rather than to the test system.

The results of the two statistical analyses conducted not only correlated well with each other, but through differential character weighting, facilitated interpretation of the data.

This work confirms the earlier report of the authors on the sensitivity and objectivity of ELISA as a means of identifying target prey consumption by polyphagous predators in a given community. Time parameters indicated by this study allow ready adaptation to field use; DFTM prey ingested by insect predators can be detected days rather than hours after feeding. The method, however, will remain largely qualitative until details of the effects of temperature and degrees of satiation on the rates of digestion can be established. Fundamental differences in the digestive processes between arachnids and insects will require clarification before a full scale quantitative as well as qualitative community study can be undertaken.

IV. TIME RELATED DECAY OF PREY ANTIGENS INGESTED BY ARBOREAL SPIDERS AS DETECTED BY ELISA

ABSTRACT

To investigate the rate of degradation of prey antigens in potential arachnid predators, individual arboreal spiders, collected from Douglas-fir from a forest in which DFTM has never been found, were fed a single first or second instar DFTM larva and held at a constant 24°C for the desired number of days. A subset of these spiders were fed either 1, 2 or 3 alternate larvae (Tribolium spp.) subsequent to DFTM consumption. Of the spiders which had a dead, apparently consumed larva in the cage, 75% were confirmed as having ingested DFTM antigens by ELISA, enzyme-linked immunosorbent assay. The same proportion of spiders were positive after being held for 12 days at 24°C. The mean number of antigen molecules present in these predators declined linearly over the 12 day period. Spiders fed one or two alternate larvae and held 5 or 7 days post-feeding closely matched the ELISA response of spiders not fed alternate larvae. Only those spiders fed 1 DFTM followed by 3 alternate larvae over a 10 day period seemed to have lost reactivity in ELISA; even then feeding by 50% of the predators could still be detected. These data are compared with similar trial feedings performed with the same prey being fed to the pentatomid predator, Podisus maculiventris.

INTRODUCTION

In recent years large investments have been made in four major studies of lepidopteran defoliators of trees: winter moth, Operophtera brumata (L.) (Varley et al. 1973); gypsy moth, Porthetria dispar (L.) (Campbell 1976);

eastern spruce budworm, Choristoneura fumiferana (Clem.) (Morris 1963); and DFTM (Mason 1976, 1981; Dahlsten et al. 1977; Mason et al. 1983; Mason and Overton 1983). Larval mortality has been identified as a major factor in maintaining these species at endemic levels, and arthropod predation has been implicated as a key regulating component. However, quantitative data on the role of various polyphagous arthropod predators on any given target-prey in the arboreal ecosystem is wanting.

Spiders are the most abundant of all arthropod predators in the western coniferous canopy (Moldenke et al. in ms. and Chapter V). They feed on all sizes of larval budworm and DFTM except the very largest (Loughton et al. 1963, D. L. Dahlsten pers. comm.) but investigation of their role in population regulation of these pests, especially at endemic levels, has not been conducted.

The development of ELISA, enzyme-linked immunosorbent assay, permits exploration of arthropod predation in arboreal systems. This assay capitalizes on the amplification inherent in enzyme substrate conversion to detect minute amounts of prey antigens in the predator gut. In feeding trials with the pentatomid, Podisus maculiventris Say, antigens from a single first instar DFTM were detected in half the predators 3 days following ingestion. The purpose of this paper is to define the degradation rate of these same antigens in DFTM-fed spiders. Knowledge of the period over which predation can be detected in these two groups of dominant arboreal arthropod predators is pertinent to an assessment of their role in population regulation of DFTM in the field.

MATERIALS AND METHODS

In spring, 1980, spiders were collected by beating lower limbs of Douglas-fir (Pseudotsuga menziesii) in

McDonald State Forest near Corvallis in which DFTM has never been collected. Dislodged specimens larger than ca. 3 mm body length were captured individually in 50x9 mm sealable plastic petri dishes. Each spider was provided water-soaked cotton and held at 24°C on a 12:12 photoperiod cycle at 70% relative humidity.

Surface-sterilized eggs of DFTM were obtained from the Goose Lake stock #144 of M. Martignoni. Larvae were reared in 15x100 mm plastic petri dishes using diet #65 (USFS Forest Sciences Laboratory, Corvallis, OR) under physical conditions identical to those used for the spiders.

Test spiders were each fed a single first or second instar DFTM larva using the following protocol. If a larva placed in the dish with a spider had not been consumed in 24 h, the DFTM was removed to a plate containing larval diet and a freshly-fed larva was introduced in its place. A freshly-fed larva was provided each spider every 24 h until one was found dead and apparently consumed. Fed spiders were held with access to water but without further food at 24°C for a maximum of 12 days. At the desired time following feeding, each was frozen and held at -20°C until tested in ELISA.

Alternate prey, Tribolium sp. larvae, approximately the size of first instar DFTM, were used to determine the effect of subsequent feeding(s) on the detectibility of DFTM antigens. Three subsamples of DFTM-fed spiders were fed one, two or three alternate prey larvae (Tribolium sp.) 3, 6 and 9 days after having consumed the single DFTM larva. One subsample of spiders consumed one Tribolium larva on day 3 (ingestion of DFTM was considered day 0) and was frozen on day 5; a second subsample fed on two Tribolium larvae, one on day 3 and a second on day 6, and was frozen on day 7; the third subsample was fed three alternate larvae, one each on days 3, 6, and 9, and frozen on day 10. Only the more available Salticidae and

Thomisidae were used in trials involving the feeding of alternate larvae.

Control spiders were maintained throughout the test period either unfed or fed on Tribolium larvae.

Each frozen specimen, held approximately 1 1/2 years following the trial feeding, was tested in duplicate via ELISA using an antiserum previously developed against DFTM. Individuals were squashed in 0.45 ml phosphate buffered saline (pH 7.2), permitted to stand for 24 h at 6°C, and the fluid portion used for ELISA. Controls consisted of antigens from unfed spiders and a dilution series of DFTM hemolymph. Substrate conversion was read spectrophotometrically.

Statistical Analysis: Two different statistical techniques were used to analyze the ELISA data. The first method employed the 95% confidence interval (mean of the control wells not containing DFTM antigens plus 2 standard deviations) to define a threshold for reactivity. A spectrophotometric value greater than this threshold was regarded as a positive reaction, i.e. a sample which contained DFTM antigens.

The second method of examining these data involved subtraction of the threshold value from each spectrophotometric reading. Each positive value thus indicated the presence of DFTM antigen, with higher values indicative of a higher concentration of reactive antigens. Day-to-day variability in time of incubation and reagent concentrations was not correctable. Since the samples tested in ELISA on any day were not ordered according to the length of time each was held post-feeding, the bias introduced in this way was probably minimal. All negative and zero values resulting from this subtraction were not included in the daily mean reactivity shown in Fig II. This mean reactivity thus represents the antigen

concentration only in those spiders which still contained DFTM antigens.

RESULTS AND DISCUSSION

No distinction was made between spiders fed first or second instar DFTM. All larvae were, in fact, very similar in size because the regime of feeding one day and not the second (the second day being spent in the predator's dish) resulted in much smaller second instars than if food had been available to them continuously. Minimal mortality of DFTM resulted from this treatment as assessed by very low mortality of larvae when not with predators.

Approximately equal numbers of Salticidae and Thomisidae, the most numerous of the families tested, were frozen on every other day of this test (roughly 10 of each family each day). Of those families represented by fewer specimens (Table IV.1), all were frozen 0 to 8 days after DFTM feeding. Selection of this regime was based on the rate of antigen decline (50% reactive on day 3, none on day 7) in the predaceous hemipteran, Podisus maculiventris Say, subjected to comparable feeding tests.

The relationship between the number of days a spider was held post-feeding and the proportion which reacted positively to DFTM antiserum in ELISA is shown in Fig IV.1. Only 75% of spiders which killed a DFTM larva on the first day of confinement (day 0) showed a positive reaction, a proportion comparable to that found in feeding trials on unstarved Podisus. Spiders taken directly from field to laboratory feeding trial include many which are satiated at the time of capture. Thus of the 46 spiders fed and frozen on the day of capture (day 0), one fourth may have instinctively killed the proffered larva without ingesting prey antigens.

No decline was evident in the proportion of spiders

Table IV.1 The number and principal species of arboreal spiders employed in this feeding trial.

spider species	Number of individuals
Salticidae	133
Metaphidippus aeneolus	
Metaphidippus harfordi	
Thomisidae	104
Philodromus rufus	
Philodromus spectabilis	
Philodromus speciosus	
Apollophanes margareta	
Oxyopidae	20
Oxyopes scalaris	
Araneidae	26
Araniella displicata	
Linyphiidae	18
Pityohyphantes rubrofasciata	
Micryphantidae	46
Micryphantid spp.	
other families	10

containing DFTM antigens over the 12 days they were held post-feeding (Fig. IV.1). This contrasts sharply with comparably-tested Podisus in which half were reactive on day 3 and virtually none on day 7. The mechanism for retention of these antigenic proteins in spiders is not known but seems to differ from that in Podisus. The digestive system of a spider consists of extensively ramifying diverticula whereas that of a Podisus resembles a simple tube. Reliance on intracellular versus extracellular digestion, especially as it concerns the loss of an epigenic site on a complex protein such as an antigen, would be likely to differ between these two systems.

Spiders subsequently fed either 1 or 2 Tribolium larvae had approximately the same DFTM reactivity as those not fed alternate prey (Fig. IV.1). However, fewer spiders reacted postively after being fed 3 alternative prey. Even after 3 alternate prey were killed and presumably consumed (as indicated by collapsed larval cuticle), previously ingested DFTM antigens were detectable in 50% of the spiders.

Regression of the spectrophotometric value against the number of days held post-feeding (Fig. IV.2) indicated a gradual, linear decline in the number of antigen molecules present over 12 days. However, the standard deviation in the spectrophotometric value of spiders tested on day 0 indicated high variability in the actual number of antigen molecules ingested. Those spiders which consumed a large quantity of antigen (high absorbance value on day 0) digested most of that material in 12 days (low absorbance on day 12) (Fig. IV.2). Those spiders which consumed relatively little antigen (low absorbance value on day 0) retained sufficient to permit its detection until the end of the test period (the same proportion of spiders are reactive on day 12 as on day 0) (Fig. IV.1).

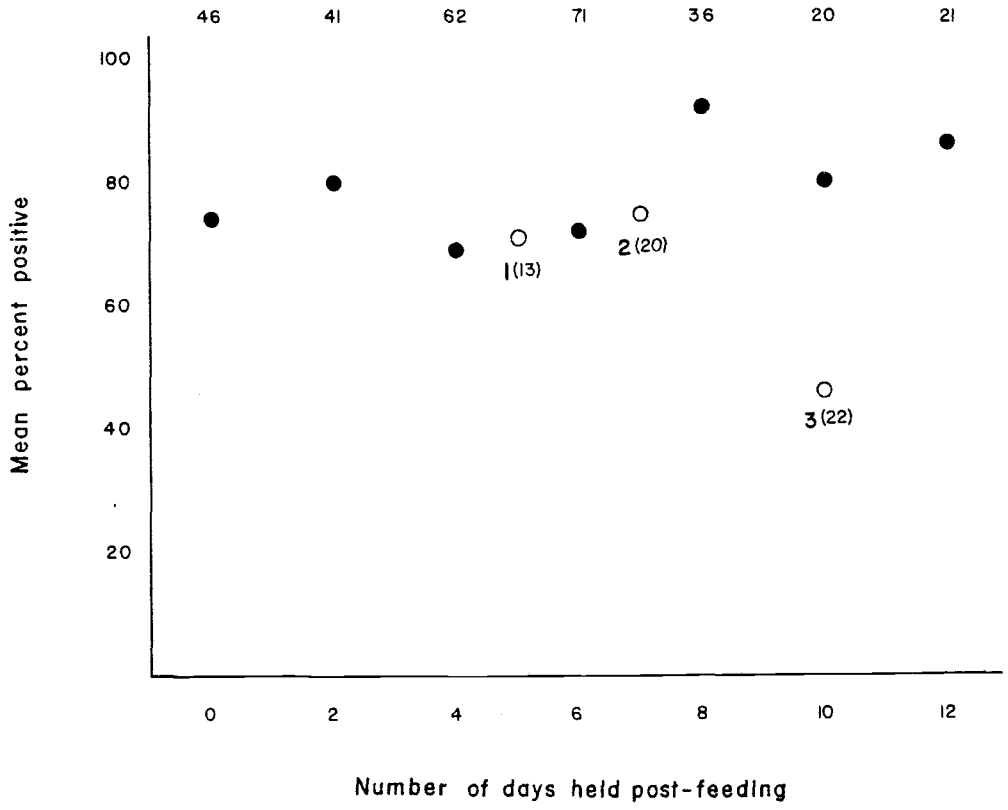


Fig. IV.1 The proportion of all spiders testing positive in ELISA (above the 95% confidence threshold) relative to the number of days each was held following ingestion of a single early instar DFTM. The black circles show the ELISA response if spiders were not fed after consuming DFTM; empty circles denote subsamples of DFTM-fed spiders which were subsequently fed 1, 2 or 3 Tribolium larvae (the number immediately under the data point indicates the number of Tribolium fed). The numbers in parentheses and those across the top of the figure indicate the numbers of spiders tested for each data point.

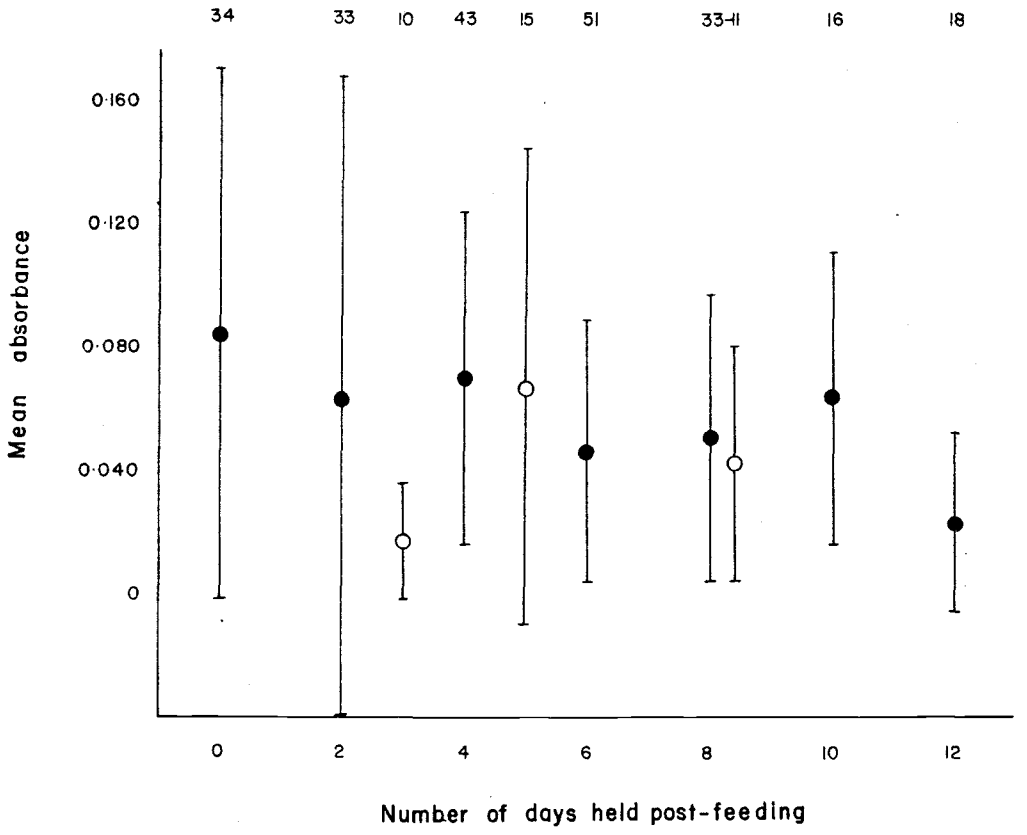


Fig. IV.2 The mean absorbance value of all spiders which had a positive ELISA response relative to the number of days each was held following ingestion of a single early instar DFTM. Since the threshold value has been subtracted from each reading, the resulting absorbance value is proportional to the number of antigen molecules remaining in the spider. Black circles are data from spiders fed one DFTM and subsequently starved. Empty circles are data from spiders fed 1, 2 or 3 Tribolium larvae following ingestion of one DFTM. The numbers across the top of the figure indicate the number of spiders tested for each data point.

Variability in the response (based either on percent positive or on mean absorbance) of Salticidae and Thomisidae negated statistically significant differences between these families. The constancy in the percent positive does allow contrast between these families and other spiders. The ranking of the spider families in this feeding trial from highest to lowest proportion of positive responses (Salticidae > Thomisidae > Micryphantidae) (Table IV.2) is the same ranking found in field-collected material (Salticidae > Thomisidae > Gnathantes). While it is possible that DFTM antigens may be more detectable in one family of spiders than in another, it seems more likely that spider families might differ in the acceptability of DFTM as food (consumatory act) once it has been killed (appetitive act).

If the ELISA value for a spider fed 1 DFTM and frozen on day 0 ($x = 0.073$, $s = 0.054$, $n = 30$ with 5 zero values omitted) is compared with that of a similarly fed and frozen Podisus tested in ELISA on the same day ($x = 0.170$, $s = 0.081$, $n = 10$), the means are found to be significantly different (Student's t test, 99% confidence, no assumption of equal population variances). Since the Podisus absorbance values average over twice those of comparably fed spiders, absorbance values resulting from tests on specimens of different taxonomic groups with an unknown feeding history, e.g. field-collected specimens, cannot now be directly compared to estimate the numbers of DFTM consumed.

CONCLUSIONS

Variability in the ELISA absorbance values of the trial-fed spiders was as great as that reported for Podisus. Prey antigens were detected in 75% of spiders fed a single early instar DFTM on day 0 as well as 12 days

Table IV.2 The percent of each family testing positive in ELISA over the first 8 days following ingestion of one DFTM. No specimens fed alternate larvae are included in these totals.

	number tested	%+
Salticidae	64	94%
Thomisidae	58	86%
Micryphantidae	46	67%
other families	85	62%

after ingestion. A linear decline in the absorbance values occurred over these 14 days. Feeding on one or two alternate prey 3 and/or 6 days after DFTM consumption did not affect the rate of digestion of DFTM antigens in spiders; consumption of 3 alternate prey over 10 days following DFTM consumption lowered the detection of DFTM antigens to 50%. Given these relatively slow rates of antigen disappearance in both the hemipteran and in spiders, an exploration of the role of arthropod predators on these target prey can be made on field populations practically and precisely. The use of ELISA should prove valuable in expanding our scant knowledge of prey selection by predators, modes of digestion and frequency of feeding in the field.

V. PREDATION BY ARTHROPODS ON EARLY INSTAR DOUGLAS-FIR TUSSOCK MOTH: A FAUNAL SURVEY OF THE WHITE FIR COMMUNITY AT BUDBURST

ABSTRACT

Early instar populations of DFTM were followed at budburst in the Sierra Nevadas, California, for 3 consecutive years during which the density declined from suboutbreak ($6.0/m^2$) to very low endemic ($0.004/m^2$). Tussock moth was considered in the context of the total arthropod community coexisting in white fir at budburst. The densities of predaceous arthropods and phytophagous species which could serve as prey for these predators were quantified. Even at suboutbreak density, tussock moth larvae did not seem to represent a significant food resource for polyphagous arboreal arthropods relative to other available prey. Due to the limited time of availability of early instar tussock moth, the extended developmental growth periods of most arboreal arthropod predators, and the relatively small biomass of these young larvae, no specialization on DFTM by arboreal polyphagous predators was observed or would be expected. No species of predator or herbivore changed density in concert with DFTM as larval populations declined 800-fold.

INTRODUCTION

Localized populations of DFTM periodically increase to outbreak densities at stressed sites in the Pacific states. Since outbreak densities result in visible defoliation (Mason 1974), or even death, of valuable host trees, an understanding of factors which normally contain DFTM at suboutbreak densities may be economically as well as scientifically profitable. Life table analyses suggest

that early instar survival is a key factor in predicting the density of each succeeding generation (Dahlsten et al. 1977; Mason 1976, 1981; Mason et al. 1983; Mason and Overton 1983).

Early instar mortality occurs as a result of unsuccessful ballooning dispersal just after hatch and as a result of predation by spiders and insects. Neither bird predation nor parasitism result in significant loss of instars I - III (Dahlsten et al. 1977; Mason 1976, 1981; Mason et al. 1983). Although the species composition of a community of arthropod predators coexisting with early instar DFTM has been documented (Dahlsten et al. 1977; Mason 1976, 1981), the mortality inflicted by each predaceous species has not been determined.

Arboreal predaceous arthropods are commonly polyphagous though some may facultatively specialize on aphids. Because of this polyphagy, the amount of predation on DFTM should theoretically be proportional to the resource DFTM represents in the community in which it occurs, i.e. one potential prey out of many simultaneously available. This paper has 2 purposes: to document the densities of dominant members of the prey-predator complex coexisting with early instar DFTM in Abies concolor and to examine the amount of predation on early instar DFTM expected in this community context.

METHODS

Field Sampling: Hatch of DFTM is synchronized with bud burst in white fir in the western U.S. Larval dispersal from egg masses laid the previous fall by the flightless females occurs via ballooning within a few days following eclosion. Sampling was timed to closely follow dispersal, i.e. when the majority of DFTM are first or early second instar. The time interval between hatch and collection of

larvae each year on all ridges but one (see below) was estimated to be 1 1/2 to 2 weeks (Wickman 1976).

White fir in the Sierra Nevada of central California was sampled for this study just after budburst in 1979, 1980 and 1981. Collections were made from three adjacent ridges in El Dorado National Forest: Iron Mountain (EIM), Plummer Ridge (EPR), and Baltic Ridge (EBR). One ridge (SSL), located in Stanislaus National Forest roughly 60 km south of the others, was sampled only in 1979 (Table V.1). Although all were mixed stands of white fir, ponderosa pine (Pinus ponderosa Laws.), incense cedar (Libocedrus decurrens Torr.) and California black oak (Quercus kelloggi Newb.), white fir was the only host to DFTM and the only tree species sampled by Dahlsten et al. (1977).

In 1979 eight plots were randomly selected along the crest of each ridge with ten 12-13 m firs tagged in each plot for use all three years (Table V.1). Each year 2 branches were cut at the bole from the lower, mid, and upper 1/3 of the crown of each tree (6 branches per tree), lowered to the ground in a bag and beaten over a ground cloth to dislodge resident arthropods. The photosynthetic area of each of the 480 branches sampled per ridge was measured (Dahlsten et al. 1979).

In 1979 and 1980, all predators greater than ca. 2 mm total body length were picked by hand from the ground cloth and frozen immediately over dry ice. Representative prey were also frozen. In 1981 all arthropods, including immatures less than 1 mm long, were collected using aspirators mounted in 7 ml scintillation vials. All soft-bodied prey, larval lepidopterans and hymenopterans, from each branch were counted each year. Aphids were recorded as present or absent. No attempt was made any year to assess the density of flying prey (principally nematoceran Diptera and parasitic Hymenoptera). Specimens were maintained at -20^{o}C until identified and

Table V.1 Number of plots from which 10 white fir were sampled in the Sierra Nevada, 1979-1981.

ridge	# plots sampled 1979	1980	1981
El Dorado National Forest			
Iron Mountain (EIM)		8	8
Plummer Ridge (EPR)	8	8	8
Baltic Ridge (EBR)	8	3	
Stanislaus National Forest			
Summit Level (SSL)	8		

serologically tested for consumption of DFTM.

Laboratory Analysis: All predators and DFTM collected in 1979 were individually weighed and identified to species in 1981. Specimens were not heat-dried in order to avoid degradation of DFTM antigens before analysis. During the 2 years these specimens remained frozen before being weighed, considerable desiccation occurred. Assuming that each specimen was desiccated to an equal degree, these weights are useful for comparative size estimation. Immature spiders were identified to species by association with adults collected during the summer and winter of 1981. No Lepidoptera, Neuroptera or syrphid larvae were reared for species identifications.

RESULTS

The density of DFTM, suboutbreak on EPR in 1979, declined 800-fold by spring, 1981 (Table V.2). An average of 3.3 DFTM were found on each branch on EPR in 1979. By 1981, so few DFTM were present that an accurate density estimate was not possible (2 DFTM found on 480 branches on EIM, 1981). At budburst on EPR, 1979, DFTM was the most abundant soft-bodied prey in white fir; in 1981 it was one of the least abundant prey.

Numerous other soft-bodied prey cohabit fir at budburst: caterpillars, mainly Geometridae and Tortricoidea, free-crawling sawfly larvae (Neodiprion spp.), sawfly larvae which web tips of new foliage (Pleroneura spp.), aphids, Psocoptera, and early instar phytophagous mirids (Table V.3). The densities of each of these taxa appeared independent of DFTM abundance except that of aphids; aphid density, estimated by the percent of branches on which they were found, was negatively correlated with DFTM density ($y = 21.0x^{-0.8}$, r^2 =

Table V.2 The density of soft-bodied insect prey collected from Abies concolor at budburst in the Sierra Nevada, California, in 1979 - 1981. Densities less than 0.1 are denoted by +; not collected is indicated by -.

density per m^2

		1979				1980			1981
		EPR	EBR	SSL	EIM	EPR	EBR	EIM	EPR
	DFTM	6.0	1.8	0.9	0.3	0.2	0.2	+	+
	Neodiprion	3.5	4.2	0.4	0.2	2.3	2.0	0.2	1.3
larval	Geometridae	0.4	0.4	0.9	0.5	0.2	0.3	1.4	1.6
	Tortricoidea	0.5	0.4	0.5	0.2	0.3	0.6	0.4	0.9
	Pleroneura	2.5	0.3	0.9	0.9	0.8	0.2	+	0.1
Pscoptera		+	+	0.4	+	+	+	0.1	0.4
phytophagous mirids		+	+	-	0.1	+	-	6.3	5.7
% of branches with aphids		15%	20%	18%	29%	24%	27%	69%	74%

0.84).

The most numerous predaceous arthropods present in white fir at budburst during all 3 years were spiders (Table V.3). Second in 2 of the 3 years were predaceous Hemiptera, principally mirids of the genera Phytocoris , Deraeocoris and Dichrooscytus. Neuroptera, ants and syrphid larvae were consistently uncommon. All of these predaceous groups accept early instar DFTM as prey though to differing degrees (Chapter VI).

Although DFTM was most abundant in 1979, actually exceeding the density of spiders greater than 2 mm total body length on EPR (DFTM density = 6.0 per m^2, spider density = 5.4 per m^2), the biomass of DFTM was never greater than one fourth that of spider biomass (Fig. V.1).

The more abundant predaceous species were generally present on all ridges each year, with the major exception of the absence of predaceous hemipterans from SSL in 1979 (Table V.4). Only 15 species of spiders and 7 species of predaceous hemipterans ever exceeded a density of 0.1 per m^2. Coccinellids were virtually the only predaceous beetles found every year.

Much of the apparent increase in density of many predators in 1981 (Table V.4) is an artifact of the different collecting technique employed that year. The density of larger spiders, adequately collected all three years, was unchanged from 1979 - 1981; only the density of immatures, many of them hatchlings, appears to increase dramatically (Table V.5).

DISCUSSION

If DFTM is an irreplaceable, vital prey to any of the predaceous arthropods, an 800-fold decline in prey density should result in a decrease in the population density of that predator. Failure to use another prey as a DFTM

Table V.3 Percent of the predaceous arthropod guild represented by each major taxon in white fir at budburst. Taxon not collected is indicated by -.

	% of total predators		
	1979	1980	1981
spiders	76	52	58
vagrant	46	33	22
web-spinners	30	19	36
predaceous insects	24	48	42
Hemiptera	13	13	25
Coleoptera	7	22	9
Neuroptera	2	8	5
ants	2	4	3
syrphid larvae	-	2	1

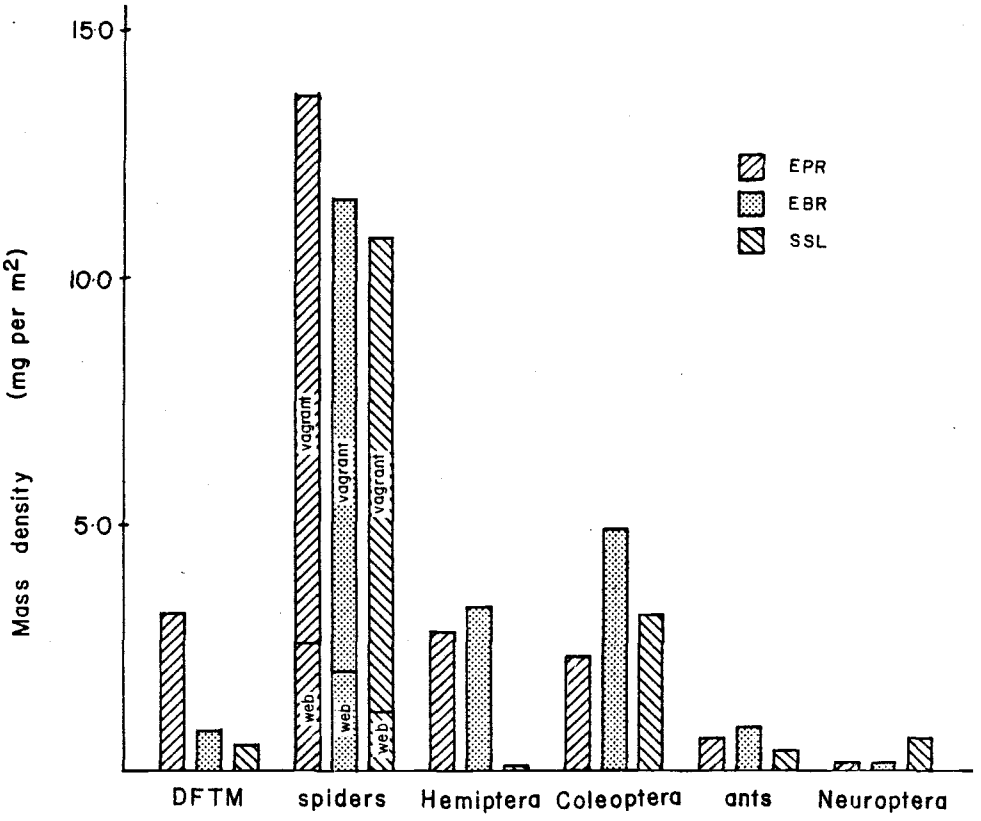


Fig. V.1 The mass density (mg per m^2 photosynthetic area) of DFTM and coexisting arthropod predators sampled at budburst from three ridges in El Dorado National Forest in 1979, the year of greatest DFTM abundance.

Table V.4 The density of arthropod predators collected from Abies concolor at budburst in the Sierra Nevada, California, in 1979 - 1981. Any taxon present at a density greater than 0.1 per m^2 on any ridge any year is included. Presence at a density lower than 0.1 per m^2 is indicated by +; no individual collected is indicated by -.

	density per m^2							
	1979			1980			1981	
	EPR	EBR	SSL	EIM	EPR	EBR	EIM	EPR
Salticidae								
Metaphidippus aeneolus	0.9	1.1	0.8	0.8	1.2	0.8	1.7	2.3
Metaphidippus harfordi	0.1	+	+	0.1	+	-	0.1	0.2
Anyphaenidae								
Anyphaena pacifica	0.5	0.4	0.4	0.6	0.5	0.1	1.9	1.9
Philodromidae								
Philodromus spectabilis	0.3	0.5	0.3	0.3	0.2	0.1	1.0	1.9
Philodromus rufus	0.3	0.4	0.2	0.3	0.4	0.3	0.6	0.9
Philodromus speciosus	0.1	0.2	0.2	+	0.1	0.1	+	0.1
Apollophanes margareta	0.6	0.2	0.4	0.3	0.6	0.4	0.4	1.0
Thomisidae								
Xysticus locuples	0.2	0.3	0.3	0.2	0.2	0.2	0.9	1.1
Linyphiidae								
Gnathantes ferosa	1.6	1.3	0.8	1.2	0.7	0.6	7.6	9.7
Pityohyphantes brachygynus	0.2	0.3	0.2	0.2	0.4	0.4	1.8	2.9
Neriene litigiosa	+	+	0.1	0.1	0.1	+	0.3	0.4
Araneidae								
Araniella displicata	0.1	0.1	0.1	+	0.1	+	0.1	0.2
Aranaeus gemmoides	+	+	0.1	+	0.1	+	0.6	0.4
Theridiidae								
Theridion neomexicanum	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1
Theridion lawrencei	-	-	-	+	+	0.1	0.2	0.3
all spiders	5.4	5.3	4.0	4.4	4.8	3.4	18.1	23.2
Hemiptera								
Phytocoris nigrifrons	0.5	0.6	+	0.3	0.5	1.2	0.3	0.6
Phytocoris calli	0.1	0.1	-	+	+	+	2.3	3.3
Deraeocoris brevis	0.3	0.4	-	0.1	+	+	0.6	0.6
Dichrooscytus abietis	+	0.1	-	0.1	0.1	0.5	0.1	0.2
Neides muticus	0.1	+	-	0.2	0.7	0.2	0.1	0.3
Tetraphleps latipennis	+	-	-	+	-	+	2.7	5.1
Podisus maculiventris	-	-	-	0.1	+	+	0.6	0.3
Coleoptera								
Mulsantina picta adults	+	+	+	0.2	0.1	0.1	0.2	0.2
larvae	0.2	0.2	+	0.4	0.5	0.6	0.9	1.2
Anatis rathvoni larvae	0.1	0.3	+	0.2	0.2	0.3	0.8	1.5
Neomysia subvittata adults	-	-	+	+	0.1	+	0.1	0.3
larvae	-	-	+	0.2	0.4	0.3	+	+
Hippodamia convergens adults	-	-	-	0.2	1.0	0.4	0.2	0.3
Neuroptera								
Agulla spp.	+	+	+	0.2	0.1	0.1	0.1	0.4
Chrysopid spp.	0.1	0.1	+	0.1	0.1	0.1	0.4	0.6
Hemerobius spp.	0.1	0.1	0.1	0.3	0.3	0.3	0.7	1.1
Hymenoptera								
Camponotus vicinus	0.1	0.1	+	0.1	0.1	0.1	0.1	0.2
Camponotus laevigatus	+	+	+	+	+	+	0.2	0.1
Formica fusca	+	+	+	0.1	0.1	+	0.3	0.2
Tapinoma sessile	+	+	-	+	0.1	0.2	0.3	0.3
Diptera								
syrphid larvae	-	-	-	0.1	0.2	0.1	0.3	0.5
all predaceous insects	1.7	2.4	0.5	3.2	5.0	3.8	12.2	18.3

Table V.5 Density (per m^2 photosynthetic area) of adult and immature spiders of the genera *Metaphidippus*, *Philodromus* and *Anyphaena* in white fir at budburst, 1979-1981.

	density per m^2		
	1979	1980	1981
Metaphidippus spp. adults	0.10	0.08	0.07
immatures	0.88	0.92	2.03
Philodromus spp. adults	0.19	0.22	0.17
immatures	0.47	0.40	1.65
Anyphaena pacifica adults	0.03	0.02	0.02
immatures	0.41	0.43	1.89

substitute could result from a need for prey of a certain size, from nutritional requirements not satisfied by alternates, or from noxious chemicals present in alternate prey. A decline in predator density might thus be caused by death of predators, by fewer eggs being laid by a fecund but nutritionally deficient female, or by predators moving into alternate host trees where acceptable prey might be more abundant. (All spider species found on fir at this time also inhabit both pine and cedar at these sites (Moldenke, unpublished data)). However, no decline in the density of any species of predator paralleling the decline in DFTM density was found (Tables V.2 and V.4).

Second instar DFTM, the largest instar found on 7 of the 8 ridges, is larger than first-year spiderlings but smaller than submatures of most vagrant spiders. Thus, if the relative biomass of predators and prey are considered, rather than just the number of individuals, most predaceous taxa would seem physically able to consume a considerable portion of the DFTM biomass (Fig. V.1) even in 1979 when DFTM was most abundant. Conversely, if this amount of biomass is all that early instar DFTM contributes to the community even when it is at high density, predators such as spiders might not find DFTM a large enough or consistent enough reward to specialize on DFTM even when DFTM was abundant.

Since DFTM was one of the most abundant prey for 2 of the 3 years even though the contributed biomass was small at budburst, a predaceous species might find it profitable to selectively prey on DFTM if it was available for a large portion of the predator's life cycle. Arboreal spiders of the Pacific states mature after 1 (Theriididae and many Linyphiidae) or frequently 2 years (Salticidae, Thomisidae, Araneidae) (Moldenke, unpublished) and all species are present in the foliage, presumably active, a large portion of the year. Arboreal predaceous hemipterans, especially

the mirids and pentatomids, are univoltine but active throughout the growing season. Coccinellids, neuropterans, syrphids and ants, all potentially multivoltine, have a similar extended activity season each year.

Most of the prey species available to these predators, in contrast, are time-limited. Tussock moth larvae, though present in the trees for 2 months in the summer, are considered potential prey to other arthropods principally during the first three instars, i.e. for only about 1 month (Mason 1976, 1981; Mason et al. 1983; Dahlsten et al. 1977). The common geometrids and tortricoids associated with Abies are characteristically univoltine and, similar to DFTM, probably are exploitable food for arthropod predators principally when small. Ants may be able to handle later instar or pupal lepidopterans (Campbell and Torgersen 1982) though they are not numerous at budburst in the white fir community of central California.

Aphids are the exception to the generality of time-limited prey. They may provide a predictable and continuous resource throughout a season upon which predators could facultatively specialize. Corresponding linearly to the increase in the proportion of branches occupied by aphids was the density of arthropods considered principally aphid associates: ants (r^2 = 0.98; y = 1.9x - 21.0), neuroptera (Hemerobiidae, Chrysopidae, Raphidiidae) (r^2 = 0.88; y = 2.8x - 25.9) and syrphid larvae (r^2 = 0.88; y = 0.6x - 7.4). There was not a strong relationship between the percent of branches on which aphids were found and the density of coccinellids (r^2 = 0.51).

CONCLUSIONS

Although early instar predation may represent a key

factor in the life cycle of DFTM, early instar DFTM does not seem to be a major resource to the community of arthropod predators at budburst. The suboutbreak density of DFTM equals that of spiders, consistently the most numerous predaceous taxon. The biomass of DFTM available at suboutbreak density, however, represents a much less significant resource. Early instar DFTM, like most prey in this ecosystem, is limited in its availability to about 1 month per year. Since all predaceous arthropods present at budburst are active continuously at least until early fall, prey other than DFTM must be utilized during the rest of the season. Most of the spider species, in fact, require 2 years to mature; DFTM constitutes a temporarily limited and unreliable food source for these species. The community of arthropods coexisting at budburst in white fir appears so bountiful and so interdependent that an 800-fold density decline in DFTM does not disturb the stability of the community as reflected in the density of any other arthropod species, either predator or prey.

VI. PREDATION BY ARTHROPODS ON EARLY INSTAR DOUGLAS-FIR TUSSOCK MOTH: A SEROLOGICAL EVALUATION OF RAPACITY

ABSTRACT

Potential arboreal arthropod predators were serologically tested, via ELISA, for consumption of DFTM. Specimens were randomly sampled at budburst from an unmanipulated ecosystem in which larval populations declined 800-fold over a 3 year study period. All species of predators accepted tussock moth larvae. The proportion of a predaceous species consuming larvae during the 2 week post-hatch period declined non-linearly with declining larval density. Salticids and predaceous hemipterans, principally mirids, consumed more larvae at a given larval density than any other predaceous guild. Evaluation of the response of predators to tussock moth larvae as one potential prey out of many available in the community revealed that all spiders accepted larvae in direct proportion to the numerical abundance of tussock moth relative to total potential prey. This pattern is expected of an unconditioned generalist predator that accepts prey as it is encountered and would result in reduced tussock moth predation when alternate prey are abundant. In contrast, the response of predaceous mirids to the abundance of tussock moth relative to all potential prey was strongly logarithmic, implying selection of DFTM as a preferred food item.

INTRODUCTION

Multi-prey, multi-predator interactions are the rule for arthropod communities. The multiplicity of potential predator-prey interactions, the bane of field ecologists

and modelers, has generally resulted in two types of analytic simplifications. One simplification used by ecologists is to focus work on only one or two predaceous or herbivorous species in the community (Horton and Wise 1983, Kajak 1978, Reichert and Tracy 1975, Putnam 1967, Pointing 1965, Loughton et al. 1963, Turnbull 1960). While this focus may yield precise answers about the species in question, seldom is the scope of investigation expanded from this base in order to yield a more encompassing perspective. This type of approach may be seriously misleading in studies of economically important pests in complex natural communities.

A second procedural simplification analyzes the community by grouping predator or prey species within broad taxonomic boundaries (Reichert and Cady 1983, Nentwig 1980, Robinson and Robinson 1970). Niche distinctions which characterize related species are not emphasized since the objective is a more general perspective. The unstated assumption is that the predator is unaffected by species distinctions. Again, this approach should be supplemented by detailed work on the uniqueness of individual species over several years during which resource abundances are varied.

Modelers have also found predator-prey interactions complex. Holling (1965) and Murdoch (1969) considered the individual predator and its satiation or prey choice. The sum of individual vagaries within an arthropod species, the effective unit in a community context, has not been examined. Analysis of interactions of multiple species has not as yet proved tractable (Hassell 1978).

Despite these methodological limitations, arthropod predation has been spotlighted as a key factor in inter-generation population dynamics of an important arboreal defoliator, DFTM. Life table analyses indicate that loss of early instars, through either dispersal or

predation, is a good predictor of the density of the subsequent generation (Dahlsten et al. 1977; Mason 1976, 1981; Mason and Overton 1983; Mason et al. 1983). Our objective was to investigate arthropod predators of early-instar DFTM in an undisturbed community where predation was identified as potentially important.

Investigation of predation by a number of different predaceous species on multiple prey species has obvious limitations. The true densities of prey and predator when predation events actually occurred are unknown. The density of each arthropod is not controlled; analysis of confounding factors in an unmanipulated community is seldom rewarding. Searching by each predator, active as in vagrant spiders or passive as in web-spinners, is assumed to coincide at least in part with the area of availability of each prey. Yet subdividing a community into small enough boxes to investigate niche overlap of individual predator and prey species yields a data bank of almost unmanageable proportions.

At endemic to suboutbreak densities, DFTM did not seem a significant portion of the prey complex available to arthropod predators at budburst (Chapter V). Polyphagous arboreal predators would not be expected to exhibit specialization on such a time-limited, biomass-limited prey. This paper examines predation that occured over the 800-fold density change in the DFTM population between 1979 and 1981 in the El Dorado National Forest of California.

METHODS

Arthropod predators and prey, collected from individual white fir (Abies concolor Lindl.) branches at budburst (Dahlsten et al. 1979; Chapter V) and immediately frozen over dry ice, were identified to species in the laboratory. All specimens were thawed for less than 5 min

before being refrozen. Following identification, representative individuals of each potential prey species were tested for cross-reactivity in ELISA using previously prepared anti-DFTM antibodies. The threshold for a positive reaction was the mean of the control wells containing Tenebrio molitor antigens plus 2 standard deviations (95% confidence interval).

In 1979 and 1980 no cross-reactions were found above a 5% error rate. In 1981, however, three species of phytophagous mirids (immatures of Orthotylus sp., Plagiognathus sp., and a third unknown species) were much more numerous than in the previous 2 years and did cross-react with anti-DFTM antibodies. Infrequent unidentified Geometridae and Tortricoidea also showed cross-reactions in 1981. Cross absorption of the antiserum was therefore employed in testing the 1981 specimens to eliminate these heterologous reactions which would otherwise be confused with DFTM consumption.

In the normal conduct of ELISA, wells are treated successively with 4 solutions: antibody to coat the well, antigen, antibody linked with enzyme (conjugate), and enzyme substrate. The mirid-induced cross-reaction was eliminated by squashing 8 mirids (2 Orthotylus , 4 Plagiognathus and 2 unknown mirids) in 45 ml conjugate diluted as necessary for direct ELISA use. This step, done at least 1 h before the conjugate was used in ELISA, allowed binding of antigens from the mirids with their complementary antibodies. When this mixture was subsequently used in ELISA, cross-reacting antibodies in the conjugate were already bound with mirid antigens in solution. Antibody used to coat the wells initially was not altered. This step eliminated reactions above the 5% error rate with all phytophagous species (Table VI.1).

Cross-absorption did not affect reactivity of anti-DFTM antibodies; spectrophotometric readings were

Table VI.1 Reduction of the heterologous positive response in 1981 to below the 5% error rate by addition of cross-reacting mirid proteins.

prey	%+ original conjugate	%+ modified conjugate
unknown mirid sp.	43	2
Orthotylus sp.	25	3
Plagiognathus sp.	14	5
geometridae	16	1
tortricoidea	12	2
aphids	3	2
Aradus sp.	0	0

comparable when DFTM hemolymph dilutions were assayed with unaltered conjugate and with conjugate previously mixed with mirid antigens. This equality suggests that antibodies reacting with phytophagous mirids were not those used to recognize DFTM antigens. Although cross-absorption of conjugate was not recognized as necessary in testing 1979 and 1980 specimens, resulting error should be minimal since these alternate prey species were not abundant; when these cross-reacting species were most dense (in 1981), only 7% of the predators were falsely positive (Table VI.1) (9%+ with original conjugate - 2%+ with modified conjugate).

In 1979, all field-collected predators were individually tested in ELISA. In 1980 and 1981, 30 specimens of each species of predator from each ridge each year were randomly selected and individually tested in ELISA. In addition, each 1981 specimen was tested against anti-DFTM antibody previously mixed with mirid antigens. All 1981 data reported herein were generated using conjugate plus mirid antigens.

Previous feeding trials established the period during which consumption of a single early instar DFTM is detectable in a representative hemipteran, Podisus, and in diverse taxa of arboreal spiders. If fed predators were held at a constant 24°C, DFTM antigens could be identified in half the trial-fed Podisus 3 days after feeding, with few detectable 7 days post-feeding. Trial-fed spiders retained DFTM antigens for at least 12 days after eating a single DFTM (constant 24°C). Since field collections were timed so that DFTM were 1 1/2 to 2 weeks post-hatch, virtually all predation by spiders should therefore have been detectable. In addition, field temperatures at budburst peaked at about 24°C with much lower temperatures during the rest of the day; lower temperatures will extend the time necessary for antigen

digestion but to an unknown extent.

Five distinct hunting guilds of spiders were distinguished for the following analyses: THOM = Thomisidae and Philodromidae, crab spiders which hunt principally by ambush; LINY = small Linyphiidae (Gnathantes ferosa and Erigoninae) that spin tiny tangled webs between subadjacent fir needles; SALT = Salticidae, jumping spiders which are active pursuit hunters; LARGE WEB = larger web-spinning spiders in the families Linyphiidae, Araneidae, and Theridiidae whose webs are often suspended between adjacent branches or branchlets; and ANYP = Anyphaenidae, a reportedly nocturnal vagrant hunter. The most abundant predaceous hemiptera (HEMI) in 1979 and 1980 were mirids in the genera Phytocoris , Deraeocoris , and Dichrooscytus.

RESULTS

All abundant species of predaceous arthropods cohabiting white fir at budburst were found to consume early instar DFTM (Table VI.2). Rarer predaceous species which were not at least 5% positive during a year when more than 10 specimens were tested were not abundant in 1979 or 1980 when DFTM was reasonably plentiful. The gnaphosid, Sergiolus montanus, and adult chrysopids were consistently uncommon; the web-spinning spider, Theridion lawrencei , and the anthocorid bug, Tetraphleps latipennis, were abundant only in 1981 when DFTM was at very low density.

Each species within a guild was analyzed individually. Variation among species within a guild was generally less than that between guilds except for the web-spinning spider, Theridion neomexicanum, which was more highly positive than other web-spinners (Table VI.2).

Although ELISA provides an objective criterion for assessing the presence of DFTM antigens, quantification of

Table VI.2 The mean percent of arboreal predaceous taxa that were positive for DFTM in 1979 and 1980 and the number of each tested. Taxa less than 5% positive were not included; 2 taxa at least 5% positive only in 1981 are denoted by *. A percentage was not calculated or averaged in the mean value reported when fewer than 10 specimens were tested from any of the 3 ridges sampled each year; in these cases + indicates that at least 1 specimen was positive and - that none were.

	% positive (# tested) 1979	1980
SALT		
Metaphidippus aeneolus	75 (330)	28 (92)
Metaphidippus harfordi	70 (16)	16 (24)
ANYP		
Anyphaena pacifica	43 (151)	31 (64)
THOM		
Philodromus spectabilis	33 (117)	17 (65)
Philodromus rufus	53 (113)	25 (89)
Philodromus speciosus	53 (47)	17 (16)
Apollophanes margareta	46 (160)	25 (84)
Xysticus locuples	28 (63)	16 (71)
LINY		
Gnathantes ferosa	32 (461)	15 (92)
LARGE WEB		
Pityohyphantes brachygynus	39 (71)	8 (88)
Araniella displicata	31 (26)	6 (26)
Theridion neomexicanum	63 (52)	23 (65)
Mallos pallidus	+ (7)	+ (10)
HEMI		
Phytocoris nigrifrons	80 (134)	66 (91)
Phytocoris calli	+ (15)	+ (14)
Deraeocoris brevis	89 (84)	55 (18)
Dichrooscytus abietis	100 (16)	79 (60)
Neides muticus	67 (16)	14 (74)
Podisus maculiventris	(0)	+ (13)
Paradacerla formicina	(0)	(0)*
Coleoptera		
Mulsantina picta adults	+ (8)	58 (64)
larvae	24 (49)	24 (88)
Anatis rathvoni adults	+ (10)	10 (17)
larvae	30 (56)	29 (81)
Neomysia subvittata adults	(0)	14 (23)
larvae	- (4)	16 (83)
Hippodamia convergens adults	(0)	7 (88)
Adalia annectans adults	- (3)	53 (17)
dermestid larvae	+ (2)	(0)*
Neuroptera		
Agulla spp. larvae	- (4)	30 (51)
Chrysopid spp. larvae	+ (12)	10 (50)
Hemerobius spp. larvae	18 (26)	8 (84)
Hymenoptera		
Camponotus laevigatus	+ (8)	10 (17)
Diptera		
syrphid larvae	(0)	10 (50)

the exact number of DFTM consumed by each predator is not possible. Variables such as size of each prey, proportion of each prey actually ingested, time elapsed since ingestion, and temperature-sensitive rate of digestion all affect the antigen titer present in any predator at any given time.

A positive ELISA (a spectrophotometric reading above the threshold value) could result from feeding on multiple DFTM larvae or on another predator which had already fed on, but not fully digested, DFTM. Since any form of food-sharing is unknown for any of these arthropod species except ants, each predator which has a positive ELISA response must have removed at least 1 DFTM from the population. Using this conservative estimate of each positive predator representing the removal of 1 DFTM, it is apparent that a large proportion of early-instar DFTM must have been removed by arthropod predators in each of the 3 years (Table VI.3). The 400 m^2 area used for comparison in Table VI.3 is roughly equal to the total foliage area sampled each year.

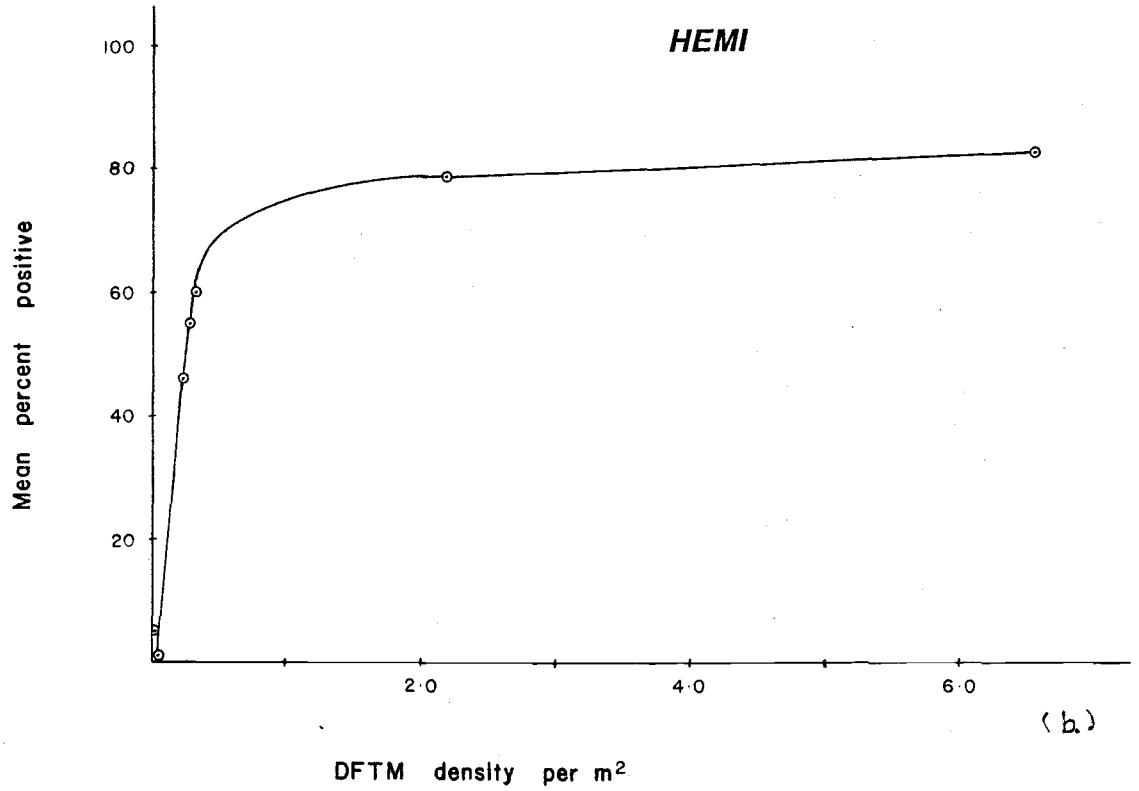
Web-spinners, except for the tiny linyphiid G. ferosa, were less numerous than vagrant hunters. The relatively low density of most web-spinners precluded subdivision of this guild by web architecture.

The proportion of SALT and predaceous Hemiptera (HEMI) that consumed DFTM increased with increasing DFTM densities (Fig. VI.1a and b) over the range of DFTM densities considered endemic (up to roughly 3 larvae per m^2) (Brookes et al. 1978). At densities above this endemic level, however, no increase in the proportion of SALT or HEMI consuming DFTM was apparent. The curves shown in Fig. VI.2 for each of the 6 functionally distinct predator guilds were sketched to fit data points such as those shown for SALT and HEMI. The proportion of each guild that has consumed DFTM increases with increasing DFTM density to a

Table VI.3 Minimum number of DFTM consumed by arthropod predators (assuming 1 positive reaction = loss of 1 larvae) and number of DFTM remaining in 400 m^2 of foliage in Sierra white fir at budburst, 1979 - 1981.

	# of DFTM consumed		
	1979	1980	1981
spiders	918	379	75
vagrant	638	288	58
web	280	91	17
predaceous insects	318	446	102
Hemiptera	251	249	76
Coleoptera	47	151	17
Neuroptera	6	35	4
ants	14	7	5
syrphid larvae	0	4	0
total removed	1236	825	177
DFTM remaining	1209	97	12

Fig. VI.1 The percent of arboreal Salticidae (a) and predaceous Hemiptera (b) which were positive for DFTM consumption as a function of early instar DFTM density. Curves are sketched to fit data points from budburst field samples from white fir, 1979-1981.


HEMI
Mean percent positive
100
80
60
40
20
2·0
4·0
6·0
(b.)
DFTM density per m²

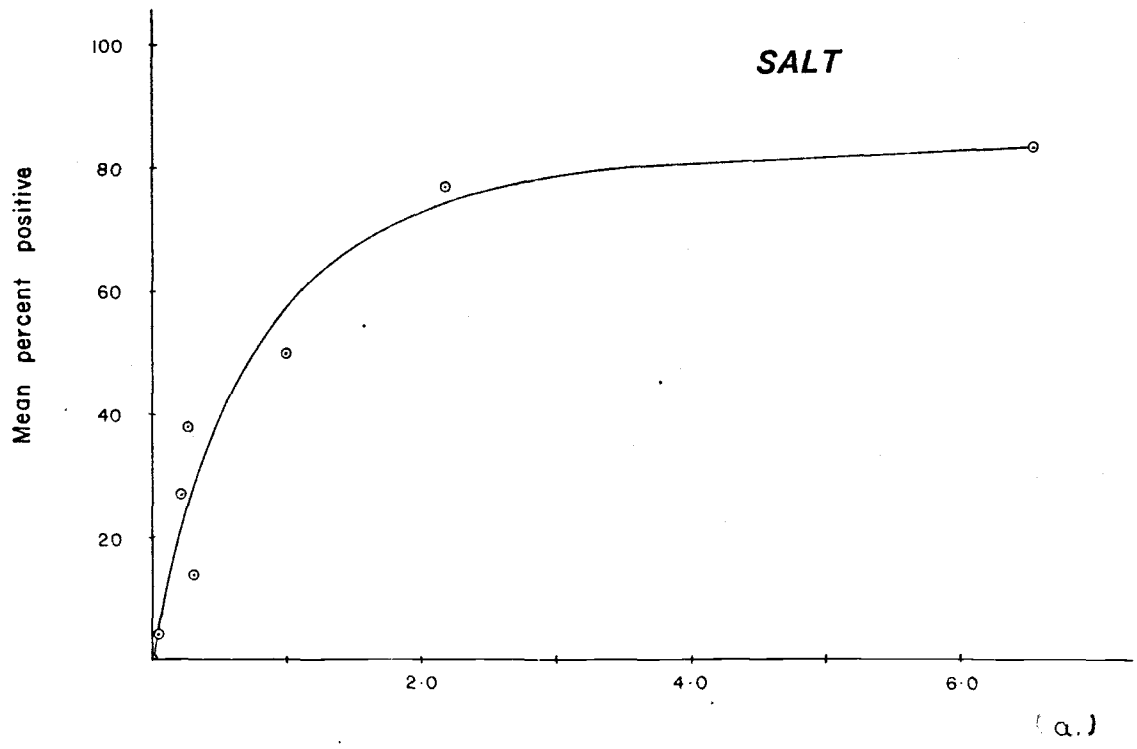

SALT
Mean percent positive
100
80
60
40
20
2·0
4·0
6·0
(a.)
DFTM density per m²

Fig. VI.1

Fig. VI.2 The percent of each guild of spiders (SALT = Salticidae, LARGE WEB = Araneidae, Theridiidae, larger Linyphiidae, ANYP = Anyphaenidae, THOM = Thomisidae and Philodromidae, LINY = the linyphiid Gnathantes ferosa and Erigoninae) and predaceous Hemiptera (HEMI) which had consumed early instar DFTM as a function of tussock moth density per m^2 photosynthetic area of white fir. Curves are sketched from data points such as those shown in Fig. VI.1.

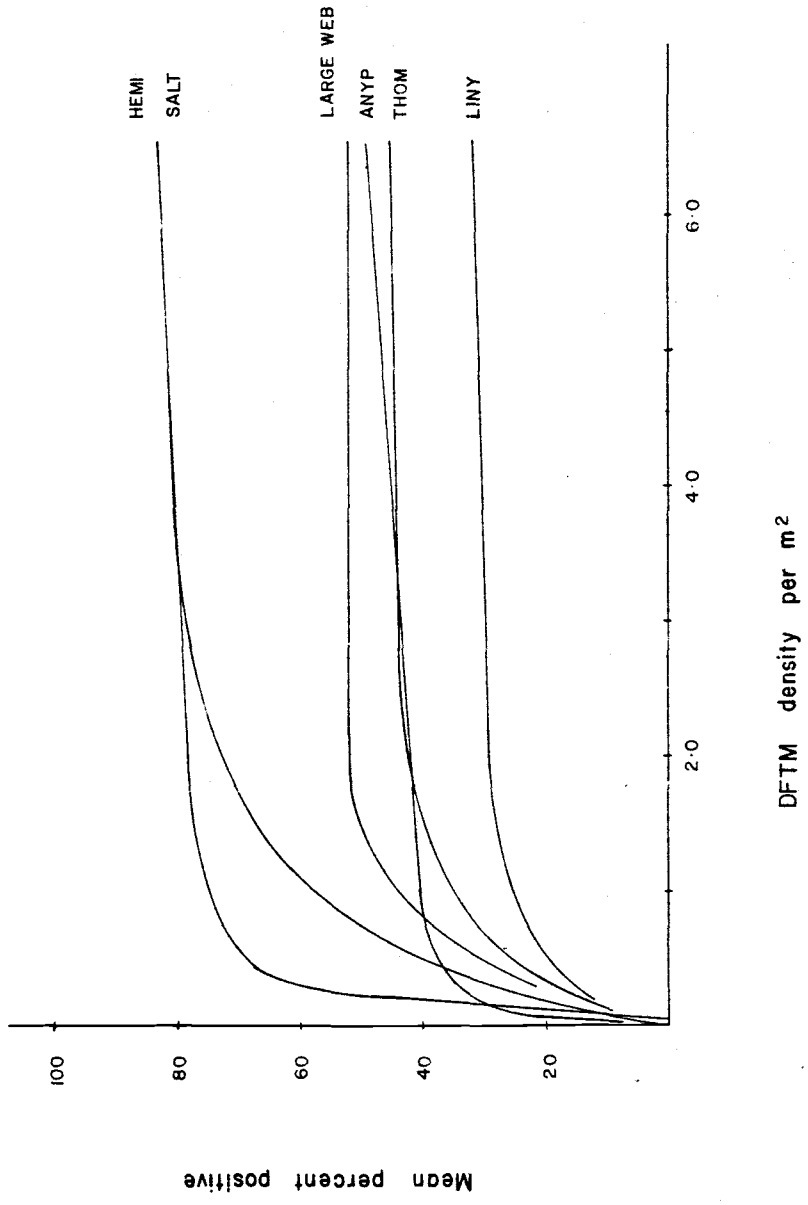

HEMI
SALT
LARGE WEB
ANYP
THOM
LINY
100
80
60
40
20
2·0
4·0
6·0
Mean percent positive
DFTM density per m²

Fig. VI.2

plateau at higher DFTM densities.

The greater the proportion of predators that are positive, the greater the probability that any individual has consumed multiple DFTM even though this multiple consumption cannot be documented serologically. The likelihood of consuming more than 1 DFTM is a direct function of the number of prey normally eaten during a 2 week period for an unconditioned predator. Since digestion of DFTM antigens occurs more rapidly in the representative hemipteran Podisus maculiventris than in arboreal spiders, multiple consumption by hemipterans is quite likely to be underestimated.

Vagrant spiders, principally in the genera Metaphidippus (Salticidae), Anyphaena (Anyphaenidae) and Philodromus (Philodromidae) consumed more DFTM than any other single predaceous group in 1979 and 1980 assuming each positive reaction represents consumption of 1 DFTM (Table VI.3). The number of DFTM consumed by each predaceous guild is, of course, strongly influenced by the density of each predator in the community. Assessment of predation by each guild with respect to its relative abundance can counter this bias. The number of DFTM each guild consumed (again assuming 1 DFTM per positive reaction) can be calculated as a fraction of the total number of early instar DFTM taken by all arthropods. Similarly, the density of each predaceous guild can be expressed as a fraction of the total predaceous arthropods concurrently present. If the proportion of DFTM consumed by a predaceous guild equals the abundance of that guild relative to all other predators, the ratio of these values will be 1.0. Predators that consume more DFTM per predator than expected on the basis of the predator's relative abundance will be above the 1.0 ratio; those that consume fewer will be below. Analysis indicates that salticids, anyphaenids and hemipterans are consuming DFTM more

frequently than predicted by their relative abundance as predators (Fig. VI.3). Decline in consumption by web-spinners relative to consumption by other predators as DFTM density delines concurs with the generally held assumption that disproporionately less dispersal ballooning of DFTM occurs at lower densities (fewer antagonistic encounters between dispersal-prone larvae).

An alternative assessment of predation considers DFTM as one prey in a community of potential prey. A spider is thought to be a non-selective predator with rejection options, i.e. a prey may be rejected as unsuitable or, if acceptable, then the rate of acceptance is directly proportional to the rate of encounter. Thus the proportion of a spider guild preying upon DFTM should reflect the abundance of DFTM relative to the total abundance of all acceptable prey. Since each acceptable prey will be taken in proportion to its rate of encounter, this correlation should hold for DFTM relative to each prey species as well as to the total acceptable prey (Fig. VI.4 a-e).

If a potential prey is rejected by a predator guild, however, this same regression should break down since consumption of an acceptable prey would not be proportional to the density of rejected prey. In an unmanipulated ecosystem such as this El Dorado forest, densities of various prey may not have changed in such a manner that all rejected prey can be identified. The only prey identified as rejected by this criterion, Neodiprion spp. refused by Salticidae, showed widely scattered data points in the regressions done in Fig. VI.4. Neodiprion are known to sequester conifer terpenoids and to be distasteful to some predators (Knerer and Atwood 1973).

To increase the clarity of Fig. VI.4, individual data points used to form each regression have not been indicated. However, each regression is based on 7-8 data points well-distributed along the entire length of the

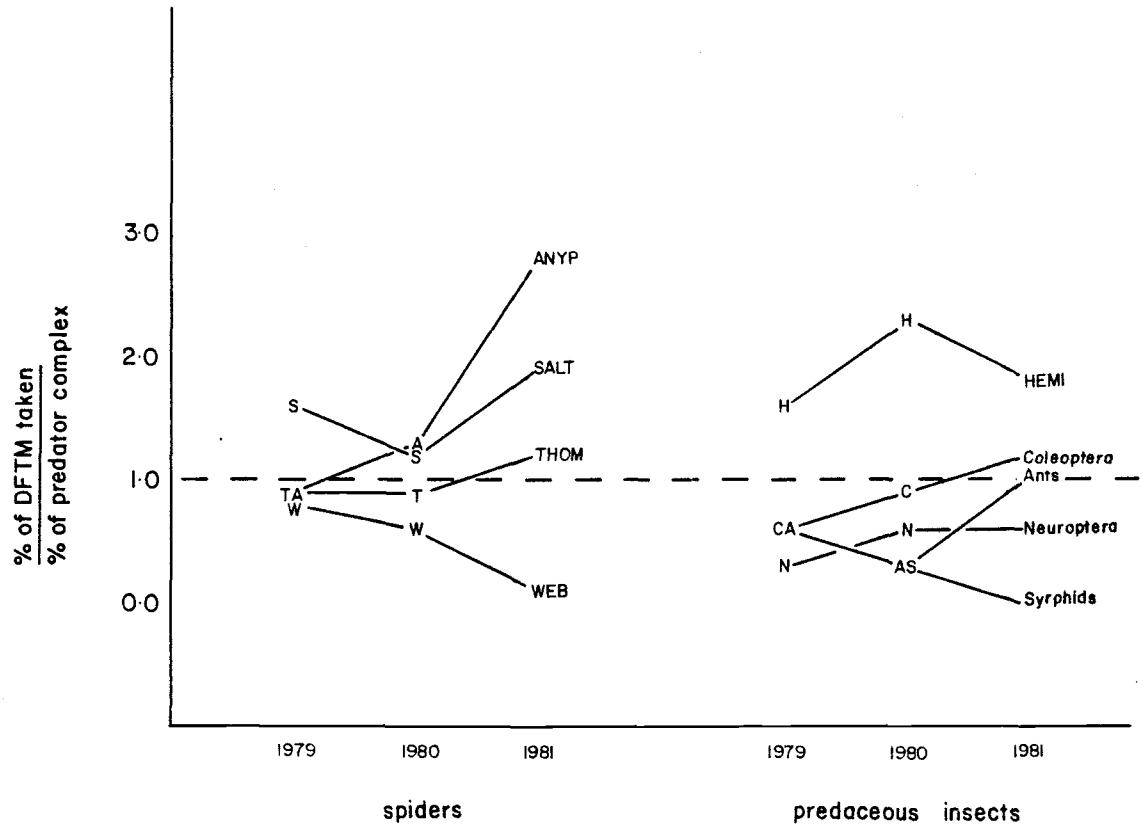


Fig. VI.3 The importance of various taxa of predators relative to total DFTM consumption and to the numerical abundance of each predaceous taxon. The value of 1.0 indicates acceptance of DFTM in exact proportion to the abundance of the predator.

Fig. VI.4 The percent of predaceous guilds positive for DFTM consumption regressed against the abundance of DFTM as a percent of total available prey. Prey names following each line indicate the prey considered in relation to DFTM; the r^2 value for the regression follows this name. Numbers of prey in an equal foliage area were used for regressions of Neodiprion spp., lepidopterans, and Pleroneura spp. (density of DFTM/density of all accepted prey). Aphids were recorded as present or absent from a branch; regressions including aphids used the percent of branches inhabited by each type of prey (percent of branches occupied by DFTM/sum of percent of branches occupied by all accepted prey).

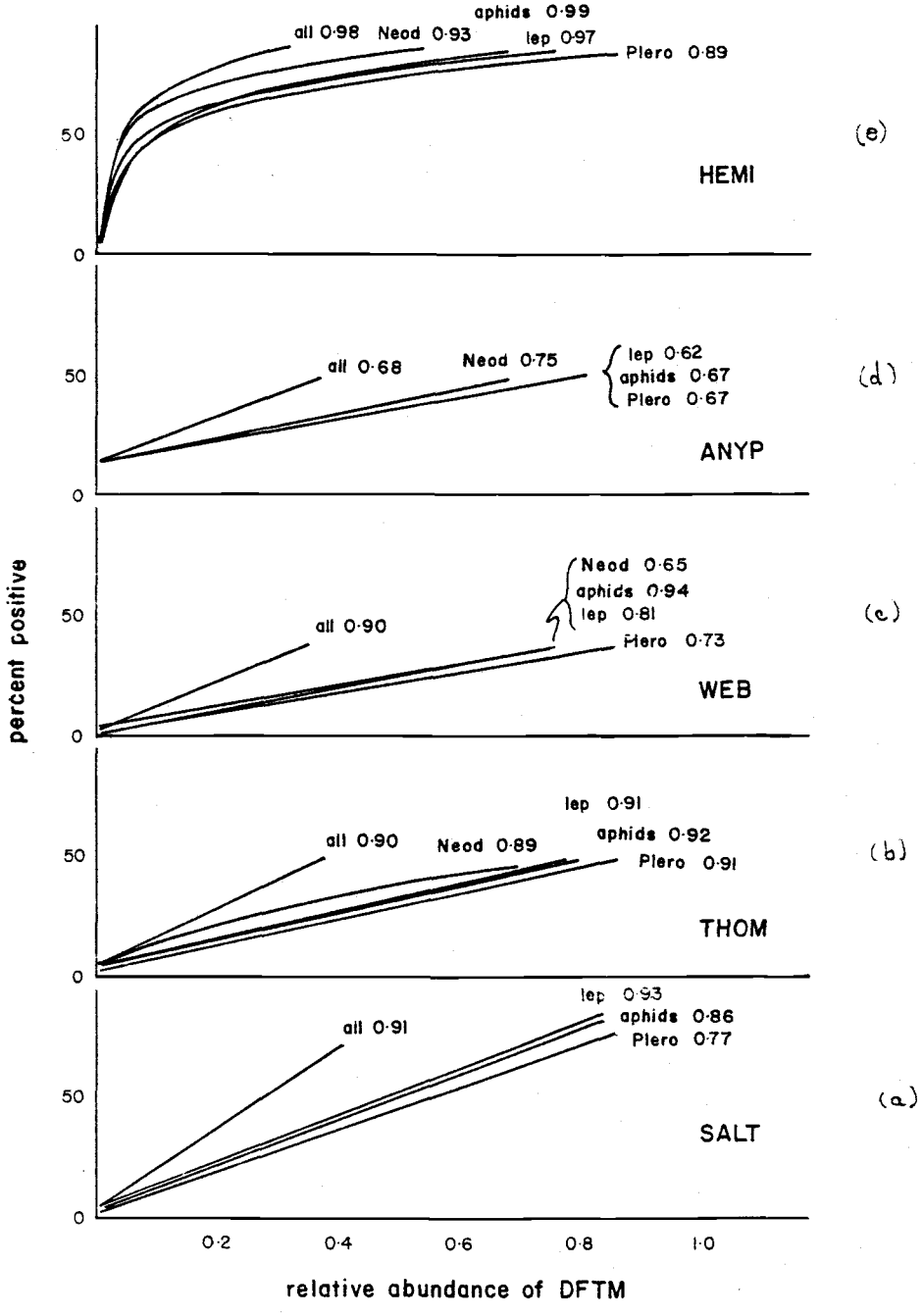

aphids 0·99
all 0·98
Neod 0·93
lep 0·97
Plero 0·89
50
0
(e)
HEMI
all 0·68
Neod 0·75
lep 0·62
aphids 0·67
Plero 0·67
(d)
ANYP
Neod 0·65
aphids 0·94
lep 0·81
all 0·90
Plero 0·73
(c)
WEB
lep 0·91
all 0·90
Neod 0·89
aphids 0·92
Plero 0·91
(b)
THOM
lep 0·93
all 0·91
aphids 0·86
Plero 0·77
(a)
SALT
percent positive
0·2
0·4
0·6
0·8
1·0
relative abundance of DFTM

Fig. VI.4

indicated line; each line is terminated by actual data points. Points are equally distributed either side of each line so that each regression is mathematically representative of the actual data.

Prior analyses of predation (Figs. VI.2 and VI.3) showed predation by SALT and HEMI to be virtually indistinguishable. The view of predation afforded by Fig. VI.4 indicates that these two predaceous groups may respond quite differently to DFTM.

DISCUSSION

Assessment of predation by any serological technique is necessarily done in retrospect. The actual densities of predators and prey at the time of capture can only be extrapolated from conditions existing at the time of sampling. For these 3 years, arthropod predation appears to account for a 2- (1235/2445) to 16-fold (177/189) decline in early instar DFTM density over a 2 week period (Table VI.3). However, DFTM density sampled over these 3 years varied 800-fold (332 to 0.4 DFTM per 100 branches). The fortuitous DFTM density change across these 3 years should minimize inaccuracies inherent in a retrospective serological technique.

All species of predaceous arthropods present at budburst accept early instar DFTM; acceptance or rejection of any other prey species has not been verified by serological analysis. If a predator rejects one species of prey, the rate of acceptance of DFTM should be independent of the number of rejected prey in the community, i.e. no correlation should be possible (such as those in Fig. VI.4). The only predator-prey association for which no regression was possible was consumption of Neodiprion by salticids. Since these sites were not manipulated, the densities of different prey species may not have varied in

such a fashion that all rejected prey can be identified by mathematical analysis alone.

Linearity in the proportion of a predaceous guild that is positive for DFTM when it is regressed against the relative abundance of DFTM of all prey (Fig. VI.4) indicates acceptance of DFTM at its rate of encounter. Even though each of these lines must pass through the origin, only data generated by field-assessed predation were used in these regressions. Different slopes may reflect different rates of encounter resulting from different hunting microhabitats. For instance, a jumping spider may forage a larger fraction of its time in the tips of the branch, the preferred DFTM niche, than does a crab spider. This may result in more frequent encounters between DFTM and a salticid than between DFTM and a crab spider, and thus a greater proportion of positive salticids relative to crab spiders, even though each guild still accepts prey at its rate of encounter.

Salticids and hemipterans were indistinguishable as potentially important predators in the first 2 analyses: predation relative to DFTM density and predation as a function of the relative abundance of the predator (Figs. VI.2 and VI.3). Jumping spiders were found to accept DFTM relative to total available prey as do all other spider guilds (Fig VI.4). Hemipterans, however, appear to prey preferentially on DFTM (Fig. VI.4 a and e). A high proportion of hemipterans fed on DFTM even when DFTM constituted only 1 or 2 bodies out of 10 potential prey. That other prey are not totally rejected is evidenced by the high r^2 values of all curves (Fig. VI.4e).

The logarithmic response of hemipterans to DFTM predicts that hemipterans will consume DFTM even when other prey is relatively much more abundant. The linearity of the salticid regression indicates that consumption of DFTM is dependent upon the density of other prey, i.e. that

fewer DFTM will be prey to salticids if aphids or other lepidoptera are also abundant.

Predation by Anyphaena is not highly correlated with the density of any other soft-bodied prey as evidenced by low r^2 values for the regression (Fig. VI.4d). These nocturnal vagrant hunters may be principally consuming prey not considered in these analyses, e.g. night-perching nematocerans, or may hunt mainly on the bole of the tree for prey not adequately sampled by bagging limbs.

Although no estimate of the actual number of DFTM consumed by each predaceous guild is possible with a serological analysis, the greater the number of positive predators, the greater the possibility that some predators have consumed more than one DFTM. Of all the predators, the already high rate of consumption of DFTM by salticids and hemipterans would be most likely to have been underestimated.

CONCLUSIONS

If predation by an individual predator described by the model developed by Murdoch (1969) were to be summed over all individuals of a species, the data in Fig. VI.2 would most likely be interpreted as a conditioned switching of polyphagous predators in response to abundant DFTM. However, when DFTM is considered as only one of many potential prey available in the community (Fig. VI.4), the actual relationship is revealed to be one of chance: all spider guilds take prey at the rate of encounter. The parsimonious explanation of chance determining predation preempts conditioned preference as a rationale. Factors dictating the response of hemipterans to DFTM are not explicable based on data now available.

A serological technique such as ELISA is not quantitative: determination of the number of DFTM consumed

by an individual predator is not possible. Interpretation of qualitative data, however, has proved fruitful since many questions do not require a knowledge of the actual number of prey consumed by each predator. Assumptions employed and predictions resulting from these interpretations are testable. Although not all questions are answered by ELISA, it does allow us to assess previously unmanageable areas of multi-predator, multi-prey natural arthropod communities and to propose mechanistic predation hypotheses that can now be pursued.

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APPENDIX

ELISA PROCEDURE

PBS: (phosphate-buffered saline)
pH 7.2 (0.01M)
stock solution: 3.15 g $NaH_2PO_4 \cdot H_2O$
20.69 g $Na_2HPO_4 \cdot 7H_2O$
400 ml H_2O
buffer: 8.5 g NaCl
40 ml stock solution
make to 1 liter with distilled water

PBS/T: PBS + 0.05% Tween 20

Carbonate buffer: pH 9.6 (0.06M)
3.81 g $NaHCO_3$
2.26 g $Na_2CO_3 \cdot H_2O$
make to 1 liter with distilled water

Preparation of IgG:

1) Combine 1.0 ml serum with 9.0 ml distilled water in a 30 ml centrifuge tube
2) Add 10 ml saturated ammonium sulfate solution
3) Leave 30-60 min at room temperature
4) Centrifuge to collect precipitate
5) Dissolve precipitate in 2 ml 1/2 strength PBS
6) Dialyze against 1/2 strength PBS 3 times, $4^{\circ}C$, 1 h minimum each time
7) Adjust OD_{280} to 1.4 (c.a. 1 mg/ml)
8) Store at $-20^{\circ}C$

Conjugation of enzyme and IgG

1) Dissolve 5 mg horseradish peroxidase (Sigma type VI, RZ = 3) in 2.0 ml of the prepared IgG
2) Add fresh glutaraldehyde solution to 0.06% final concentration; mix well
3) Leave 4 h at room temperature
4) Dialyze 3 times against 500 ml PBS to remove glutaraldehyde
5) Add bovine serum albumin (BSA) to a concentration of 5 mg/ml; store at 4°C (do not freeze)

Coating of microtitration plate

1) Rinse plate thoroughly 3 times with distilled water and finish with a rinse using carbonate buffer. Shake out solutions between rinses.
2) Add optimal dilution of antibody in carbonate buffer (250 µl/well)
3) Allow to stand at least 4 h at 30°C in high humidity
4) Store in high humidity at 4°C (leaving the IgG solution in the wells)

Substrate

Prepare immediately before use: 1% O-phenylenediamine in methanol

Combine: 20 ml distilled water
0.2 ml O-phenylenediamine solution
6 µl of a 1/10 dilution of H_2O_2

Note: This substrate is photoreactive.

Execution of ELISA

1) Shake antibody from wells and rinse plate 4 times with PBS/T
2) Dispense antigens diluted in PBS/T (200 µl/well)
3) Incubate 30 min at 30°C
4) Shake antigen from wells and rinse 4 times with PBS/T
5) Dispense conjugate diluted in PBS/T (200 µl/well)
6) Incubate 30 min at 30°C
7) Rinse 4 times as before
8) Add substrate (200 µl/well)
9) Incubate in the dark 30 min at 30°C
10) Add 9N H SO (25 µl/well) to stop the reaction
11) Read in a spectrophotometer at 490 nm

Appendix Fig. Sequential steps in ELISA: 1) coating the surface of the microtitration well with antibodies, 2) complexing the surface-bound antibodies with homologous antigen, 3) linkage of homologous antibodies carrying an enzyme marker with bound antigens, and 4) conversion of enzyme substrate at a rate proportional to the number of bound enzyme molecules.

Appendix Fig.

ENZYME-LINKED IMMUNOSORBENT ASSAY