INFLUENCE OF *NOSEMA FUMIFERANAE* (MICROSPORA) INFECTION ON FLIGHT OF THE OBLIQUEBANDED LEAFROLLER (LEPIDOPTERA: TORTRICIDAE): A FLIGHT MILL STUDY

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MARK GARDINER

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Name: MARK GEORGE GARDINER

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Examining Committee:

Chairman: Dr. A. T. Beckenbach, Associate Professor

Dr. B. D. Roitberg, Associate Professor, Senior Supervisor,
Dept. of Biological Sciences, SFU

Dr. M. Mackauer, Professor,
Dept. of Biological Sciences, SFU

Dr. J. E. Cossentine, Research Scientist,
Agriculture Canada

Dr. J. H. Borden, Professor,
Dept. of Biological Sciences, Public Examiner

Date Approved November 30, 1990.

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Author:

(signature)

MARK GARDINER

(name)

DEC 7, 1990.

(date)

ABSTRACT

A rotary flight mill system was utilized to assess the effects of the pathogen, Nosema fumiferanae (Thomson), on the flight performance of the obliquebanded leafroller, Choristoneura rosaceana (Harris). A comparison of flight by healthy and N. fumiferanae-infected, virgin male leafrollers revealed that diseased moths were capable of flying substantial distances, although in some instances, healthy moths flew significantly greater distances. Diseased moths flew on average, 17 percent slower than healthy moths. Flight performance, based on distance flown and flight velocity, was not significantly correlated with N. fumiferanae spore load. Body weight, wing area, and wing load were generally similar for healthy and infected moths. Overall, N. fumiferanae had less influence on flight performance than was anticipated from longevity and histology studies. The results of this study suggest that the presence of N. fumiferanae in wild male obliquebanded leafrollers may not significantly interfere with mate and habitat finding, two important activities that involve flight. From a biocontrol perspective, infected leafrollers might beneficially transmit N. fumiferanae to neighboring healthy leafroller populations.

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I. LITERATURE REVIEW

In recent years, there has been increasing interest in using pathogens in insect pest management (Maddox 1982; Miller et al. 1983; Falcon 1985; Morris et al. 1986; Brooks 1988). These pathogens include viruses, bacteria, fungi, protozoa and nematodes. Entomopathogens are but one of many biorational approaches to insect management being pursued in an effort to eliminate, or at least substantially reduce, the use of conventional chemical insecticides.

Most research on entomopathogens has concentrated on virulent bacteria and viruses, pathogens that kill their hosts rapidly. Some, such as Bacillus thuringiensis (Berliner) (Morris et al. 1986) and many of the viruses (Falcon 1985), are very efficacious. Currently, B. thuringiensis and 2 forest insect viruses are registered for insect control in Canada; others, such as the granulosis virus of Cydia pomonella (L.), are presently being evaluated. As of 1984, 4 insect viruses and 4 species of Bacillus were registered in the United States and 8 additional viruses were under development (Falcon 1985). Typically, these lethal pathogens are utilized and applied like conventional chemical insecticides.

Some entomopathogens, particularly many protozoans, cause little or no acute mortality to infected insects. Instead protozoan infections usually produce chronic, debilitating diseases (Canning 1982; Wilson 1982; Brooks 1988). While a few protozoans, such as Nosema locustae Canning, show promise, their value in insect management remains to be proven. While they have generally been overlooked because of a lack of virulence, recent research has suggested that protozoans can play an important role in the dynamics of their hosts' populations (Anderson and May 1980; Henry

1981; Andreadis 1984; Maddox 1982). Because population reductions from protozoan infections occur slowly, their use is of questionable value in crop protection if economic damage thresholds are low (Canning 1982). Therefore, sublethal pathogens show most promise in controlling insects associated with forest and forage crops, as well as range land where economic damage thresholds are generally high and immediate or total insect control is not feasible (Maddox 1982; Brooks 1986).

Canning (1982) believes that nonlethal protozoa will be useful as part of a "two or more component strategy" in which a microsporidian would be used in conjunction with either another entomopathogen or a chemical biocide. This belief originates from research that has demonstrated that insects afflicted by a chronic disease are often more susceptible to chemical insecticides as well as other insect pathogens than healthy insects.

Of the entomopathogenic protozoa, microsporidia show the greatest potential in insect management (Canning 1982; Maddox 1987; Brooks 1988). Microsporidia are spore-producing, intracellular pathogens (Vavra 1976) which are commonly found in insects (Weiser 1976). The familiar but outdated classification system has placed many unrelated pathogens in the phylum Protozoa. However, a recent reclassification has elevated many taxa to the phylum status. Once considered a class of the phylum Protozoa, the microsporidia are now classified in a separate phylum, the Microspora (Levine et al. 1980).

The host ranges of microsporidia are not well understood. The difficulty associated with identifying microsporidia (Kellen 1974) has contributed to this confusion. Species such as N. pyrausta (Paillot) and

N. carpocapsae Paillot are known to infect only one insect host, Ostrinia nubilalis (Hübner) and C. pomonella respectively. N. fumiferanae (Thomson), initially believed to be restricted to C. fumiferana (Clemens) and other closely related species of Choristoneura (Thomson 1958b), was subsequently shown to infect several species from different lepidopteran families in laboratory assays (Wilson 1981). Pleistophora schrubergi (Zwölfer) has also been recovered from a wide variety of lepidopteran species (Kaya 1973). Microsporidia are also known to infect hymenopterous parasitoids associated with their primary hosts (Own and Brook 1986).

The consequences of a microsporidian infection are variable and depend on the particular host-pathogen association (McLaughlin 1971), the developmental stage of the host (Weiser 1976, Cossentine and Gardiner 1990), the amount of pathogen inoculum ingested (Cossentine and Gardiner 1990), and various environmental stress factors (Siegel et al. 1986). While a few microsporidia, such as Vairimorpha necatrix (Kramer), are extremely virulent, causing rapid death of their host (Canning 1982), most produce chronic, sublethal infections. Examples of sublethal microsporidian diseases include N. pyrausta infection of O. nublialis (Windels et al. 1976) and N. fumiferanae infection of C. fumiferana (Thomson 1958a; Wilson 1981). In both of the above Nosema-host interactions, some mortality occurs when young larvae are subjected to high inoculum doses, but many larvae complete metamorphosis and become diseased adults. Malone and Wigley (1981), examined the effects of N. carpocapsae on C. pomonella and observed no larval mortality at inoculum doses tested. In this instance, all Nosema infections were chronic and sublethal.

Transmission of microsporidia can occur perorally (Weiser 1976), congenitally (Fine 1987), and sometimes through pathogen-contaminated parasitoids (Weiser 1976; Own and Brooks 1986). The congenital pathway, known as vertical transmission, involves the direct infection of offspring by diseased parents. Maternal transmission, where the pathogen may occur in or on the insect's egg is the most frequently documented mode of vertical transmission and appears to be common in microsporidian infections (Canning 1982). The existence of paternal vertical transmission, whereby sperm or accessory fluids are contaminated with the pathogen, is less certain. Thomson (1958b) indicated that male C. fumiferana infected with N. fumiferanae sometimes transmitted the pathogen to their offspring. Subsequent work by Wilson (1984), however, failed to substantiate Thomson's results, and Malone and Wigley (1981) were unable to demonstrate paternal vertical transmission of N. carpocapsae. Therefore, because most research has focused on maternal vertical transmission, it is not known whether paternal transmission is unusual or has simply been overlooked.

All other modes of transmission, both between and within generations, that do not involve the direct transfer of pathogen, from parent to offspring, are considered horizontal transmission (Canning 1982). Typically horizontal transmission involves the ingestion of spore-contaminated food by susceptible insects, with spores originating from contaminated larval feces as well as larval and adult cadavers. While some insects, eg. grasshoppers (Brooks 1988), can become infected as adults, most insects acquire microsporidian infections as larvae, and susceptibility to infection usually decreases as larvae mature (Wilson

1974). As mentioned previously, some parasitoids are known to transmit microsporidia, but the importance of this mode of transmission is not known.

Once a microsporidian has invaded its host, the infection spreads to various tissues. While the types of tissue infected may vary depending on the host and the pathogen, commonly infected tissues include the midgut, Malpighian tubules, muscle, reproductive organs, and the fat body (Weiser 1976). Infection proceeds at the expense of energy the insect would normally commit to its own development and reproduction (Khan and Selman 1989).

Although there is interest in using sublethal pathogens in insect management, surprisingly little research has evaluated how chronic infections debilitate their hosts. Most work has been limited to assessing, for a few host-pathogen associations, how microsporidia infections influence development, reproduction and longevity. Often diseased larvae grow more slowly (Thomson 1958a), resulting in smaller adults (Mercer and Wigley 1987) that typically produce fewer offspring, during a shortened lifespan (Windels et al. 1976; Malone and Wigley 1981; Armstrong and Bass 1986; Brooks 1986; Mercer and Wigley 1987; Bauer and Nordin 1989). Pathogen-infected females may also experience reduced mating success, measured as the frequency of mating (Armstrong and Bass 1986; Mercer and Wigley 1987). Finally, Malone and Wigley (1981) observed that healthy female C. pomonella produced fewer eggs when mated with N. carpocapsae-infected males than when mated with healthy males.

Only recently has the influence of disease on adult behavior and mobility been investigated (Forsse 1987; Sweeney and McLean 1987; Humphry

and Linit 1989; Sanders and Wilson 1990), yet the need for research in these areas seems obvious. For many insects, particularly moths, flight is very important in both mate acquisition as well as movement among habitats. If the presence of disease adversely affected an insect's ability to fly, both the mating success of the insect as well as the potential dispersal of the pathogen could be reduced. Presumeably research on this subject has been avoided because quantitative evaluation of insect flight presents many obstacles. While the ideal approach would involve examining flight under natural, field conditions, this is rarely possible. The recapture rate for most insects is very low and is inversely proportional to the species' flight range (Hocking 1953). It would also be difficult, if not impossible, to gather data on potential flight range or flight velocities in the wild. Finally, in a situation that involved diseased insects, release of the pathogen into the environment may not be appropriate. Often therefore, it is necessary to analyze insect flight in the laboratory.

A review of the literature suggests that there are 2 distinct laboratory approaches to the evaluation of insect flight: the wind tunnel and tethered flight systems. Further, there appear to be 2 distinct approaches to tethered flight: stationary flight systems and rotary flight mills.

A wind tunnel consists of a chamber through which air flows and insects are allowed to fly unrestrained (eg. Cardé and Hagaman 1979). In some wind tunnels, visually patterned, moving floors are incorporated to provide motion feedback information to flying insects (Cardé and Hagaman 1979). Insects that rely on visual cues to assess flight velocity can be

maintained in flight, within the moving air of the wind tunnel, by adjusting the velocity of the floor to the insect's intended flight velocity. Typically, the observed flight activity is recorded manually but sometimes video cameras are utilized.

As previously mentioned, two distinct tethered flight systems have commonly been utilized: a stationary type, whereby an insect is attached to the end of a light, hinged wire that pivots vertically, allowing the insect to takeoff and land (eg. Gatehouse and Hackett 1980; Naranjo 1990), and a rotary flight mill, consisting of a horizontal rotor that pivots about a vertical axis allowing an insect to move freely through a horizontal circular path (eg. Chambers and O'Connell 1969; Resurreccion et al. 1988). The insect is attached to one end of the rotor, usually by a small wire harness glued to the insect's thorax. Ideally, a flight mill rotor features low aerodynamic and frictional drag (Hocking 1953). Both types of tethered-flight systems may be interfaced with microcomputers that allow continuous, prolonged data acquisition (Clarke et al. 1984).

Selection of a particular approach to the laboratory evaluation of insect flight depends on the aspect(s) of flight to be investigated. To examine flight orientation to a stimulus, such as a pheromone source, a wind tunnel is most appropriate. However, if the objective is to evaluate the flight ability of a insect over a long time interval and derive data on flight velocity and distance flown, then the rotary flight mill is useful. When the focus of a study is on flight activity patterns rather than actual distance and velocity data, the stationary flight mill, on which the insect is free to takeoff and land, would likely produce more

realistic results.

While all of the above approaches are artifical to some extent and may not provide a true picture of what an insect actually does in nature, they do provide some indication of an insect's flight capacity. They are particularly useful in comparative studies, where the effects of various treatments on flight ability are of interest. Examples include assessing the effects of gamma radiation (used to sterilize insects sexually) (Sharpe and Chambers 1976), pheromone components (Cardé and Hagaman 1979), and disease on insect flight performance (Townson 1970; Sweeney and McLean 1987; Humphry and Linit 1989). Laboratory flight systems have also been effectively utilized to assess the influence of age, sex or mated status on the flight activity of a species (Sharpe et al. 1975; Foley 1985) and to explain the difference in dispersal patterns between two closely related insect species (Roitberg 1988).

II. INTRODUCTION

The obliquebanded leafroller (OBLR), C. rosaceana (Harris), is indigenous to North America and occurs throughout much of the continent (Gangavalli and AliNiazee 1985). The OBLR is polyphagous and feeds primarily on plants in the family Rosaceae (Reissig 1978). A noted pest of many agricultural crops (Gillespie 1981), the OBLR has recently become a serious pest of pome fruits in British Columbia (Madsen et al. 1984) and filberts (AliNiazee 1986) in the Pacific Northwest. While most of the larval feeding damage in apples is restricted to foliage, larvae also feed on developing apples, resulting in surface scarring. Currently, researchers at Agriculture Canada in Summerland, British Columbia, are conducting research into biorational methods of control for the OBLR as part of an effort to reduce the use of conventional chemical insecticides.

My project was part of a larger study evaluating the potential of the microsporidian N. fumiferanae (Microsporida: Nosematidae) as a biocontrol agent for managing the OBLR. N. fumiferanae was derived from the eastern spruce budworm, C. fumiferana, a close relative of the OBLR. No microsporidian has yet been identified in the OBLR. Initial studies showed that N. fumiferanae readily infects the OBLR in the laboratory (Cossentine and Gardiner 1990). While early instar larvae, subjected to high doses of N. fumiferanae, suffered high mortality, most fourth and fifth instar larvae survived to become diseased adults.

Many studies have demonstrated that chronic sublethal infections can have debilitating effects on a wide variety of adult insect life history traits although no information is available for N. fumiferanae-infected OBLR. This project investigates how a N. fumiferanae infection influences

the flight capability of adult OBLR.

Flight can be of great importance to an insect. In the OBLR, males utilize flight to search for and follow pheromone plumes to sexually receptive females, thus male mating success depends on flight ability. Also, the OBLR will likely use flight to find and exploit new habitats or recolonize agricultural land (from adjacent wild habitat) after being temporarily controlled.

In addition to the obvious effects on mating success, altered flight activity of N. fumiferanae-infected OBLR could have important ramifications for the value of the pathogen in biocontrol. Natural dispersal of the pathogen would depend, in part, on flight activity of infected moths. For example, Thomson (1958b) observed that some male and all female N. fumiferanae-infected eastern spruce budworm, transmitted the pathogen to their offspring. If similar modes of transmission occur in the OBLR, both male and female OBLR, infected with N. fumiferanae, are potential vectors of the pathogen. Diseased moths capable of sustained flight might introduce the pathogen into healthy OBLR populations.

There is good reason to believe that the presence of a Nosema infection would detrimentally affect the flight activity of the OBLR. In many host-pathogen associations involving microsporidia, both muscle and fat tissue are infected. This has been observed for the eastern spruce budworm infected with N. fumiferanae. Also, Thomson (1958a) suggested that invasion of the midgut of eastern spruce budworm larvae, by N. fumiferanae, interfered with the assimilation of energy reserves by the fat body. Khan and Selmann (1989) state that "most of the pathogenic effects observed in the host-pathogen systems are related directly or

indirectly to the depletion of resources in the fat body of insects". Many Lepidoptera derive energy for flight primarily from the fat body (Bailey 1975); therefore any reduction in fat body size could mean that less energy was available for flight. Moths such as the OBLR are believed to rely on food reserves acquired as larvae, with adult feeding being of little or no importance.

Research examining the effects of disease on flight is rare. Studies involving non-microsporidian pathogens have examined the effects of mammalian pathogens vectored by mosquitoes, and nematode infections in two species of beetle. The studies involving mosquitoes showed that the pathogens had a detrimental impact on many aspects of flight (Townson 1970; Hockmeyer et al. 1975; Schiefer et al. 1977). In contrast, the nematode infections had no significant effect on the flight of their beetle hosts (Forsse 1987; Humphry and Linit 1989).

Two flight studies involving microsporidia have recently been published. Sweeny and McLean (1987), discovered that male western spruce budworm, C. occidentalis Freeman, infected with a Nosema sp. were significantly less responsive to pheromone in wind tunnel assays than were healthy males. Electro-antennogram experiments revealed that antennal sensory cells of diseased moths were detecting the pheromone, leading these authors to speculate that either moths were unable to process the sensory information or else that the diseased moths were physically unable to fly. Sanders and Wilson (1990), examined the flight response of N. fumiferanae-infected male eastern spruce budworm to calling females. Employing a wind tunnel with a moving floor that kept the moths in sustained flight, Sanders and Wilson (1990) observed no significant

difference between the duration of flights for healthy and diseased moths. However, the flights averaged less than 5 min and likely did not include the total flight time a wild moth would normally spend in search of females. In this study, the authors failed to mention what percentage of the moths tested actually failed to respond to the pheromone. Thus, at present there is no consensus on the effect of Nosema infection on flight in moths.

The current study utilizes a rotary flight mill to compare the flight ability of healthy and N. fumiferanae-infected OBLR over long time intervals by assessing flight velocities and distances flown. Preliminary studies assessed the flight of healthy male and female OBLR. The sex exhibiting the greatest flight activity was then used to compare healthy and diseased moths. This seemed appropriate because I reasoned that a difference would be most easily detected in the sex exhibiting the greatest flight activity. Ideally, the effect of N. fumiferanae infection should have been assessed for both sexes.

Before flight studies began, the effects of harnessing moths was assessed by comparing the longevity of moths handled and treated with glue, with unmanipulated control moths. Before assessing the impact of N. fumiferanae infection on flight, the longevity of healthy and diseased male moths was compared to assess the impact of the infection and to ensure that diseased moths lived sufficiently long to mate and reproduce. Finally, N. fumiferanae infected OBLR were examined histologically to determine if the pathogen infected tissues important to flight.

III. METHODS AND MATERIALS

1. Rearing system for Choristoneura rosaceana

OBLR were obtained from a laboratory colony maintained at the Summerland Research Station that had been established in 1985 from an Okanagan Valley, British Columbia, orchard population. Larvae were reared in sealed, 28 ml plastic cups, on a pinto bean-based diet (Appendix). The OBLR rearing chamber was maintained at 24.0±0.5° C, with a 16:8 (L:D) photoregime, provided by fluorescent lights (Sylvania™, cool white F48T12/CW/VHO).

Eggs were obtained by hanging waxed paper strips (oviposition substrate) in 20 l white plastic buckets containing approximately 70 pairs of moths. Adults in these oviposition cages were provided with a 10 % sucrose solution, in dental wicks.

Eggs were held in sealed 28 ml plastic cups, containing moistened filter paper, until they hatched. Neonates were placed individually, with the aid of a fine camel-hair brush, on fresh diet within 5 h (usually 1-3 h) of hatching.

2. Propagation of Nosema fumiferanae

Nosema fumiferanae was isolated from the eastern spruce budworm, C. fumiferana. N. fumiferanae spores were provided by G. Wilson of the Forest Pest Management Institute, Forestry Canada, Sault Ste. Marie, Ontario.

To maintain a supply of fresh infective spores, N. fumiferanae was propagated in OBLR larvae. Third instar OBLR larvae were fed fresh diet,

coated with N. fumiferanae spores. The number of Nosema spores applied to the diet was expressed as the number of spores/mm^2 of diet surface. Spores were applied to the diet surface in 0.2 ml of distilled water. After the water had evaporated, larvae were placed in the diet cups. Because the quantity of spore suspension and the diet surface area (per cup) were fixed, the spore concentration on the diet surface was controlled by varying the concentration of the spore suspension. The concentration of Nosema spore suspensions was determined with a hemacytometer (Fig. 1) following the procedure outlined by Cantwell (1970).

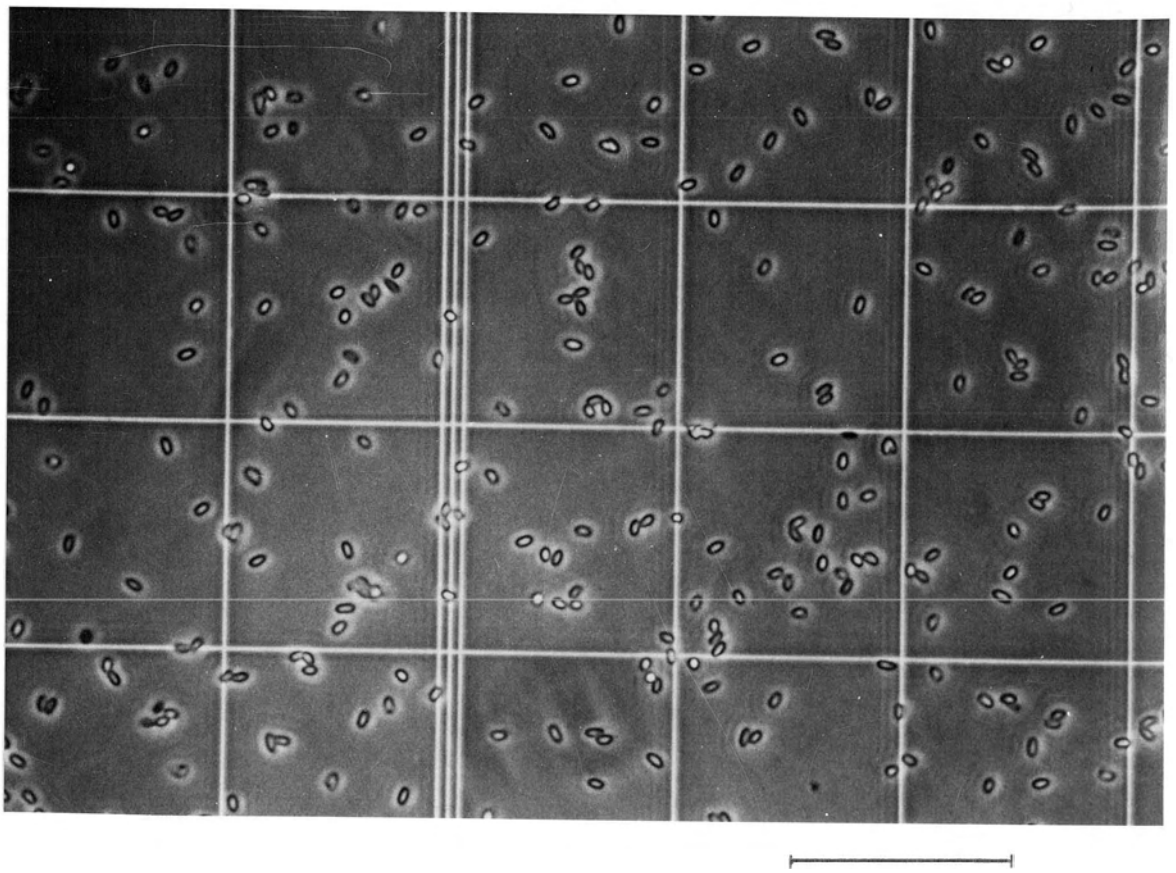
Nosema spores were collected from diseased 4th and 5th instar larvae by blending the larvae in distilled water, in a tissue homogenizing tube. This crude suspension was purified by filtering it through numerous layers of cheese cloth to remove the larger debris. The resulting solution was further purified by centrifugation at 2000 rpm for 6-10 min. The centrifugation process was repeated 2-3 times, with the supernatant being removed and the spore pellet resuspended in distilled water between centrifugations. The result was a suitably pure pellet of N. fumiferanae spores.

The purified spore material was stored in distilled water with penicillin, at either 4° or -10° C. Spores used for experiments were never more than 2 months old.

3. Histological examination of N. fumiferanae-infected adult male C. rosaceana

Infected moths were obtained from 4th and 5th instar larvae that had

Fig. 1. N. fumiferanae spores viewed against the hemacytometer grid used to determine concentrations of spore suspensions. Bar = 0.05 mm.



been exposed to diet treated with Nosema spores at a concentration of 1000-3000 spores/mm^2 of diet surface. To confirm if N. fumiferanae infected adult OBLR tissues involved in flight (eg. thoracic flight muscle and fat body), diseased male OBLR were examined histologically. After removing the wings and legs, a freshly killed adult was divided into head, thorax and abdomen and fixed in Bouin's fixative. To compensate for the hydrophobic nature of moth scales, a small quantity of Triton-X 100^{TM} was added to the fixative. Once fixed, the tissue was infiltrated with paraffin, sectioned, and stained with Giemsa stain.

4. Effects of N. fumiferanae infection on longevity of adult male C. rosaceana

A longevity experiment was conducted in an environmental chamber at 24.0±0.5° C, 16:8 (L:D) photoregime (Sylvania^{TM} cool white fluorescent lights) and 50±5% RH.

Diseased moths were produced by transferring healthy, recently molted, 5th instar larvae to diet treated with 1000-3000 spores/mm^2 of diet surface. Healthy OBLR were reared similarly, without exposure to the pathogen. All larvae were reared individually, in 28 ml plastic cups with lids. Pupae were transferred individually, to empty 28 ml cups.

Upon emergence (pupae checked twice daily), healthy and diseased moths were kept individually in 196 ml clear plastic cups. Each cup contained a dental wick, saturated with distilled water. Presence of pathogen in potentially diseased moths (those derived from larvae exposed to pathogen) was confirmed by examining their meconium at 250x magnification for Nosema spores.

Health of the moths was assessed daily at approximately the same time. Dead, diseased moths were air dried for at least 1 wk, weighed, and homogenized individually in water to determine the number of Nosema spores/mg body weight.

Longevity experiments for healthy and diseased moths were not conducted simultaneously; however, the source of moths, type of diet and experimental conditions were identical.

Mean lifespans were compared by a t-test.

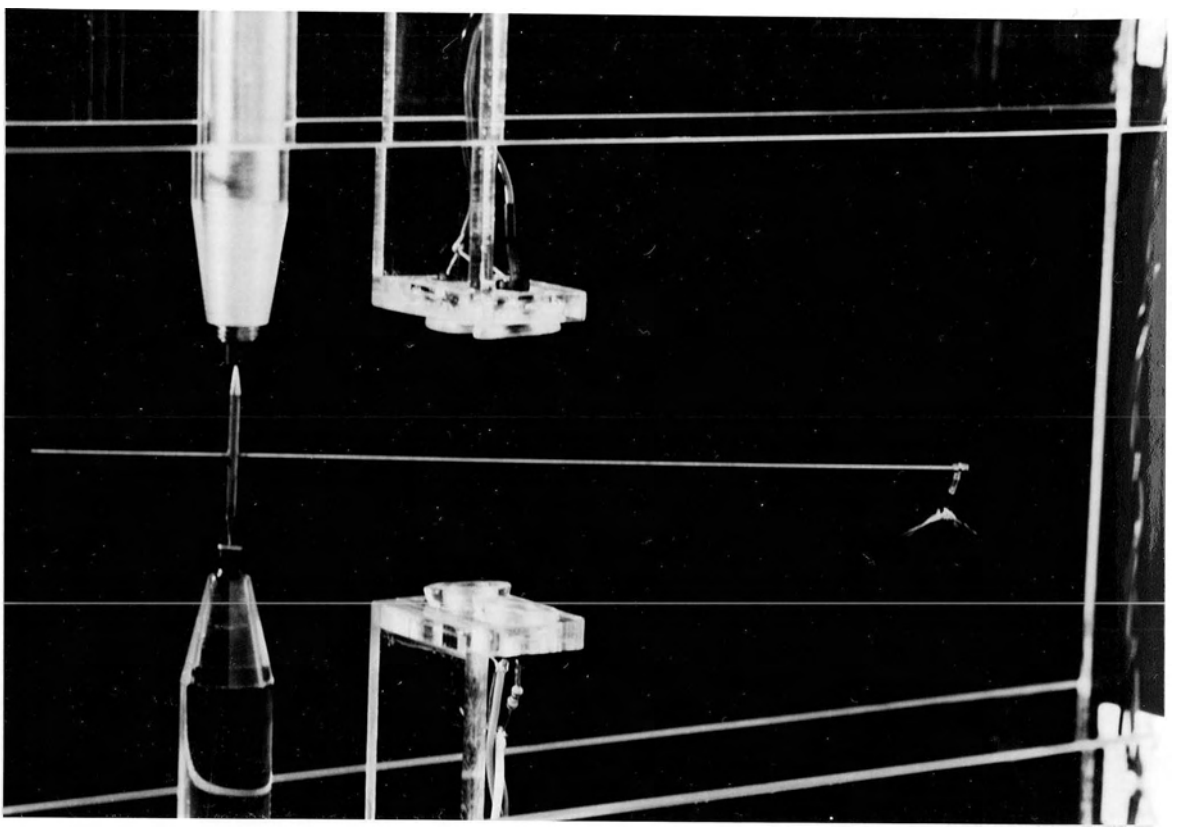
5. Analysis of adult C. rosaceana flight ability

a) Description of flight mill system

The flight capability of healthy and N. fumiferanae-infected OBLR moths was assessed using a rotary flight mill system. Each mill consisted of a horizontal rotor attached to a vertical pivot that was suspended between two small magnets (Fig. 2). The mill design was based on a version described by Chambers and O'Connell (1969). Moths were attached to one end of the mill rotor and, when flying, described a horizontal, circular path of approximately 1 m. An infrared photo emitter-detector system detected the rotations of the mill rotor when a moth was flying.

Four flight mills were interfaced with an IBM-compatible microcomputer that recorded time and mill rotor rotation data at 30 sec intervals. The computer also recorded the initiation and termination of each individual flight (a flight was considered over when the mill rotor took longer than 5 s to complete 1 rotation). Data compiled for each mill in a given session, were readily converted to ASCII format. As well, data recorded for each mill were automatically summarized in terms of number of flights

Fig. 2. Close-up photograph of a male OBLR harnessed to the end of a mill rotor. Components of the mill include the magnetic pivot system, and to the right, the photo emitter-detector unit responsible for detecting revolutions of the mill rotor as the moth flies.



and distance flown per flight. The hardware[1] and software[2] that enabled the flight mills to be interfaced with a microcomputer were patterned after the system employed by Clarke et al. (1984).

The flight mills were housed in a 2.0 x 2.5 m walk-in environmental chamber (Fig. 3). The mills were located on an 80 x 145 cm table with 40 cm high side walls that: 1) restricted potential air movement that might interfere with mill operation, 2) and provided uniform visual surroundings for the moths. A radial pattern, consisting of 6 black lines was centered under each mill to provide the flying moths with a visual reference. The flight chamber was maintained at 24.0±0.5° C and 50±5% RH. During the photophase, light was provided by a bank of 8 fluorescent bulbs (Sylvania™, cool white F48T12/CW/VHO) positioned 45 cm above the flight mills. Thirty min of reduced light intensity (6 fluorescent bulbs), preceded and followed a 7.0 h scotophase.

The following procedure was used to attach a moth to a flight mill rotor. Moths were immobilized by placing them in a 0-1° C walk-in refrigerator for approximately 10 min. Once immobilized, a moth's movement was further restricted by placing it in a narrow trough formed by folding a file card. While viewing the moth through a 8x magnifier, a fine camel-hair brush was used to remove scales from the dorsum of the

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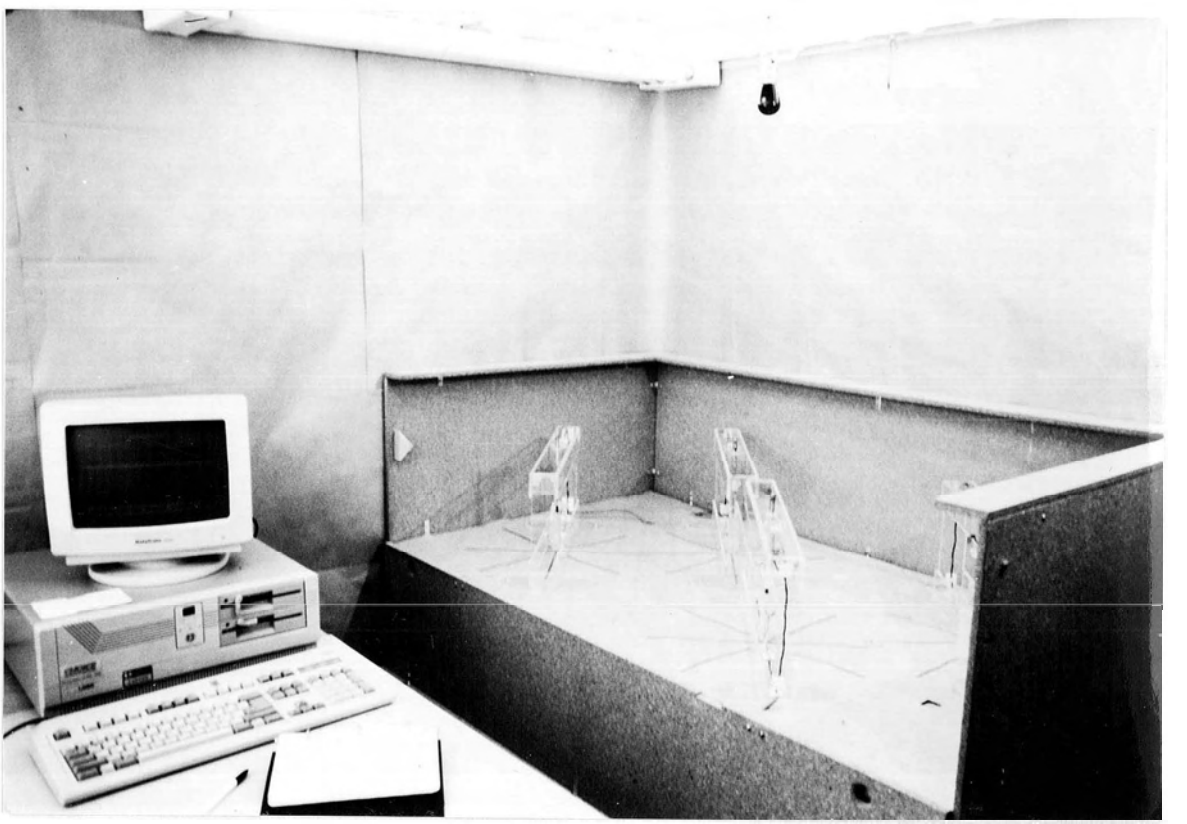
[1] The circuit board that allowed flight mills to be interfaced with a computer was made by I. Tarasoff of the Simon Fraser University Electronics Workshop, Burnaby, British Columbia.

[2] Computer software was developed and provided by H.E.L. Maw, Agriculture Canada, Ottawa, Ontario.

Fig. 3. Photograph of a rotary flight mill system housed in an environmental chamber. The 4 flight mills situated on the table are interfaced with the microcomputer though a RS232 serial port.



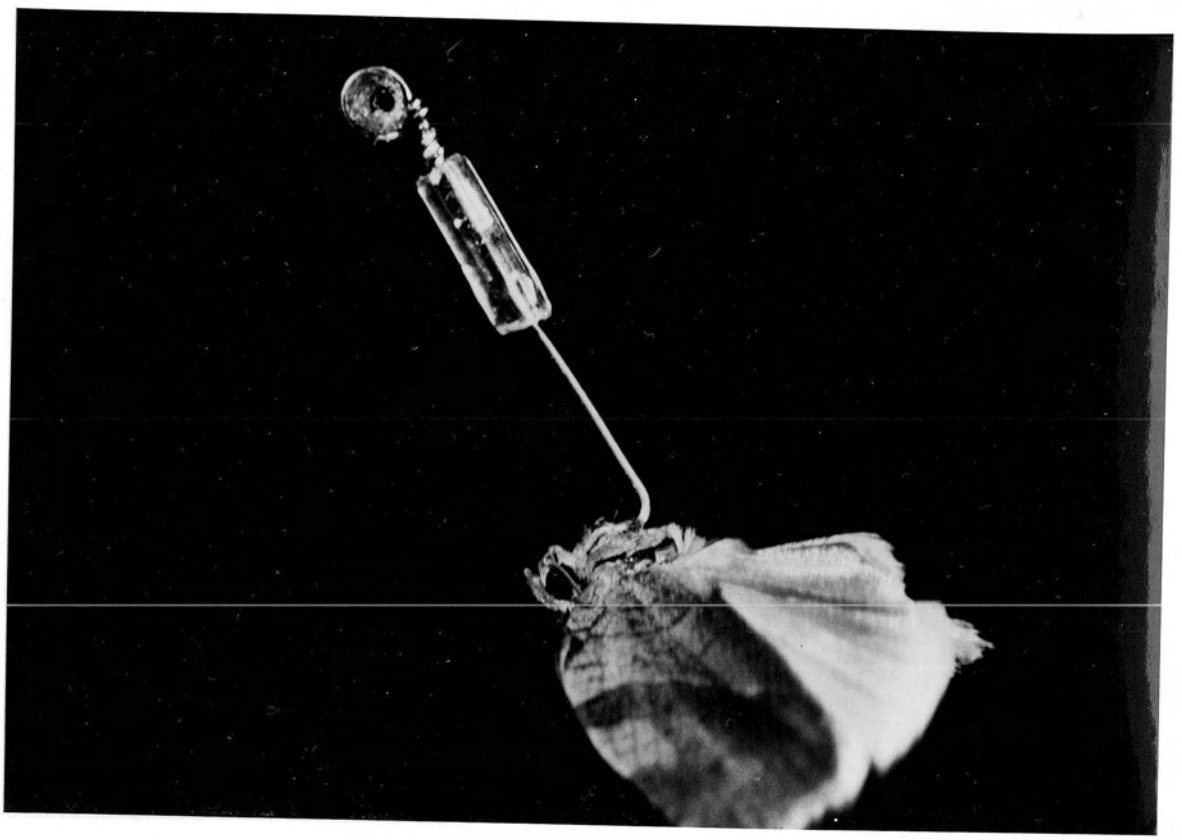
mesothorax. Once the scales were removed, exposing an area of clean, smooth cuticle, a wire harness was glued to the moth's mesothorax with Lepages™ contact cement. The harness was made from fine chromium wire and consisted of a small loop (ca 1.5 mm in diameter), which was glued to the moth, and a straight portion (ca 7.0 mm) bent at approximately 80° to the terminal loop that attached to the mill rotor (Fig. 4).

Harnessed moths were placed on the inverted bottom of a small petri dish and covered with a 28 ml plastic cup, where they remained until being attached to a flight mill. Moths were weighed (to nearest 0.1 mg) and held at 24° C for at least 20 min before being flown.

A moth was attached to a mill arm by picking it up by the harness, with a forceps, and pushing the straight portion of the harness into the hollow end of a short section of wire insulation fixed to the end of a mill rotor. Linkages in the harness attachment system were quickly adjusted so that the moth was longitudinally perpendicular to mill arm with wings level and a head-up pitch attitude of approximately 5°. The mill arm was then re-attached to the mill housing and data recording was initiated. During attachment (usually < 20 sec), moths were held without tarsal contact and typically beat their wings. If after re-attaching the mill arm to the mill, a moth was not flying, it was subjected to a maximum of 2 tarsal releases using a clean glass slide. If the moth was still not flying, it was left in this flightless state. Moths were free to fly or rest for the remainder of the flight test period, as they chose.

After a moth completed its test period, it was removed from the mill, placed in a labelled 28 ml cup, and stored in the freezer. Later, wing areas and wing lengths, as well as spore concentration for diseased moths

Fig. 4. Detailed view of a harnessed OBLR in flight, showing attachment of wire harness to both the moth's thorax and the mill rotor.



were determined. Forewing lengths, measured from the tegula to wing tip, were determined to the nearest 0.1 mm using an ocular micrometer (Miller 1977). To determine wing areas, the right forewing and hindwing were removed from each moth and mounted between glass slides. The image of the wings was then magnified 8x using a photographic enlarger. These enlarged images were then assessed utilizing a video camera interfaced with a computerized image analysis system.

b) Chronic effects of glue on adult C. rosaceana

After evaluating numerous glues, LEPAGES™ contact cement was chosen to attach flight harnesses to the OBLR. The chronic effects of the glue and handling on the OBLR was assessed by comparing the lifespan of moths exposed to contact cement with control moths not exposed to the glue. Twelve male and 10 female moths were tested per treatment.

Two-day-old moths were immobilized by placing them in a -5° C freezer for 2-3 min. After removing scales from the dorsum of the mesothorax with a fine camel-hair brush, a small quantity of contact cement was applied to the exposed cuticle. Control moths received no treatment. All moths were held, individually, in 196 ml cups at 25° C and a 16:8 (L:D) photoregime (cool white fluorescent bulbs). RH was maintained at 50±5%. Moths were examined each afternoon.

Mean lifespans of control and glue-treated moths, for each sex, were not significantly different (t-test, $P > 0.05$), suggesting that the handling and gluing procedure had no serious detrimental effect on the moths.

c) Flight of healthy male and female C. rosaceana

For details of OBLR rearing and the flight mill system, refer to

sections 1 and 5(a), respectively.

In 1989, 66 healthy, 1-2 day-old, virgin male OBLR were flown on the rotary flight mills. Before flight tests, moths were kept individually in 196 ml clear plastic cups and provided with water. Flights began 1.5 to 3.0 h before scotophase and were terminated after 5 h. In 1990, 22 healthy, 1-2 day-old, virgin female OBLR were flown. Flights began 2.5 to 6.5 h before scotophase and were terminated after 90 min. Weight prior to flight, as well as forewing length and wing area, were determined for each moth.

Data gathered included flight velocity, length of first flight, length of longest flight and total distance flown during test period. A flight was defined as at least 50 m of continuous flying. The number of flights/moth/test period were also determined.

Influence of moth size on flight velocity and distance flown was assessed by linear correlation analysis. Correlation analysis was also employed to evaluate how well the total distance flown by male moths in 5 h could be predicted by their performance in the first 90 min of the test period.

Flight performance of male and female moths was evaluated. The distance flown by males and females in 90 min was compared. Because flights were often truncated, flight distances were compared using a non-parametric Mann-Whitney test. Flight distances, summarized categorically, were compared using a Chi-square test. Mean flight velocities, wing areas and body weights were compared using the t-test. An alpha value of 0.05 was used in all statistical tests.

d) Flight of healthy and N. fumiferanae-infected male C. rosaceana

For details of OBLR rearing, production of N. fumiferanae infected moths, and the flight mill system, refer to sections 1, 2, and 5(a), respectively.

In 1989, the flight performance of 16 healthy and 21 N. fumiferanae infected male OBLR was evaluated on the rotary flight mill system. During 1990, an additional 21 healthy and 16 diseased moths were flown. All moths were unmated and 1-2 days old. Prior to each flight test, moths were kept individually in 196 ml clear plastic cups and provided with water. Diseased moths were produced by exposing 5^{th} instar larvae to diet treated with 1000-3000 Nosema spores/mm^2 of surface. Not all OBLR larvae subjected to N. fumiferanae became diseased adults. Although the percentage varied with each batch of larvae, only about 10 % of the larvae developed into infected adults. Initial assays conducted in 1987 (Cossentine and Gardiner 1990), using similar procedures, typically resulted in nearly 100 percent infection of adults with little larval mortality. I have no plausible explanation for this difference. Presence of pathogen, in a potentially diseased moth, was determined, prior to flight, by microscopic examination of the meconium for Nosema spores.

All moths were tested on the flight mill for 90 min. Tests took place from 0.5 to 7.0 h prior to scotophase. Healthy and diseased moths were randomly assigned to flight mills and flown simultaneously.

Following test termination, moths were stored at -8° C. Later, forewing length and wing area was measured for each moth. In addition, for diseased moths, N. fumiferanae spore concentration was determined. This procedure involved homogenizing individual moths (minus wings and

legs) in a known quantity of water. Then, following the technique described by Cantwell (1970), spore concentrations were determined utilizing a hemocytometer. By knowing the spore concentration in a known volume of water and the original weight of the moth, the number of spores per milligram of moth body weight was calculated.

Data gathered include flight velocity, length of first flight, length of longest flight and total distance flown during 90 min test period. A flight was defined as at least 10 m of continuous flying. The number of flights/moth/test period was also examined.

Flight performance of healthy and diseased moths were compared using above mentioned criteria. Flight distances were compared using a non-parametric Mann-Whitney test. A Chi-square test was used to compare categorical flight distance data. Mean flight velocities, wing areas, body weights and wing loads were compared using a t-test. Correlation analysis was used to evaluate the influence of N. fumiferanae spore load on the various flight variables. An alpha value of 0.05 was used in all tests.

IV. RESULTS

1. Histological examination of N. fumiferanae-infected adult male C. rosaceana

Microscopic examination of Giemsa-stained tissue from 4 adult male OBLR revealed that the fat body was extensively infected with N. fumiferanae. In contrast to tissue from healthy OBLR, where the cell structure of the fat body was readily observed, no cell structure was discernible in the fat body of diseased moths. Also, clusters of Nosema spores were observed in thoracic muscle tissue (Fig. 5). N. fumiferanae may have been present in other tissues but this was not investigated.

2. Effects of N. fumiferanae infection on longevity of adult male C. rosaceana

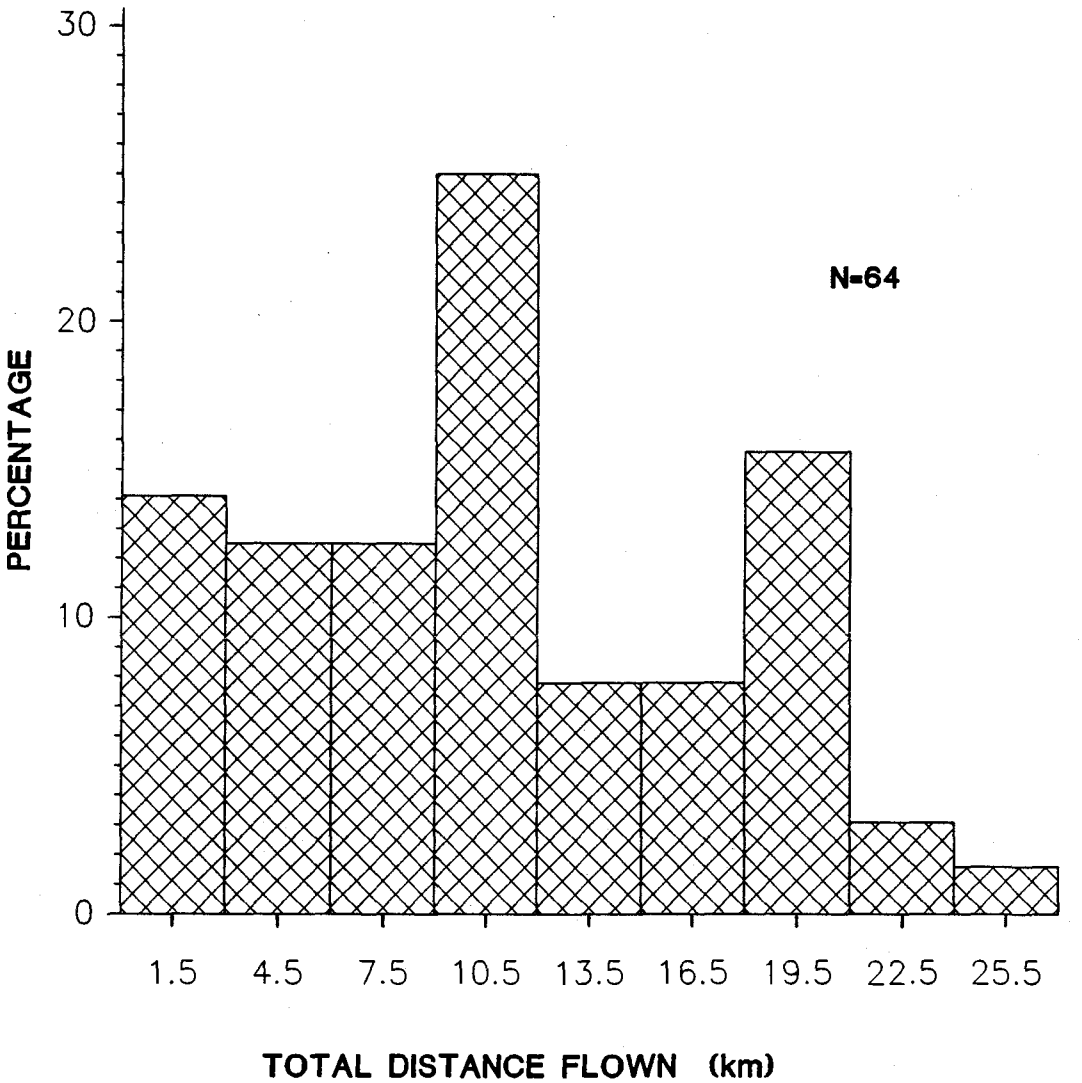
Diseased OBLR, with a mean lifespan of 6.9 d (SE=0.74, N=7), died significantly ($P < 0.05$) sooner than the healthy moths, which lived an average of 13.2 d (SE=0.74, N=20). The lifespan of diseased moths ranged from 5 to 10 d, whereas healthy moths lived from 9 to 17 d. Dry weight N. fumiferanae spore concentrations averaged 1.48×10^7 spores/mg body weight (SE=1.12×10^6, N=7).

3. Flight of healthy male and female C. rosaceana

Most male OBLR flew considerable distances in the 5 h test period (Fig. 6). Fifty percent of the moths flew more than 10 km; only 2 males failed to fly. Fifty-eight of the 66 moths (87.9%) made at least one continuous flight over 1 km. Sustained flights greater than 5 km were

Fig. 5. Photograph illustrating Giemsa-stained male OBLR thoracic muscle tissue with clusters of black N. fumiferanae spores. Bar = 0.05 mm.

Fig. 6. Total distance (km) flown by healthy male OBLR in 5 h test period. This figure does not include 2 moths that failed to complete at least 1 flight longer than 50 m.


30
20
10
0
PERCENTAGE
N=64
1.5
4.5
7.5
10.5
13.5
16.5
19.5
22.5
25.5
TOTAL DISTANCE FLOWN (km)

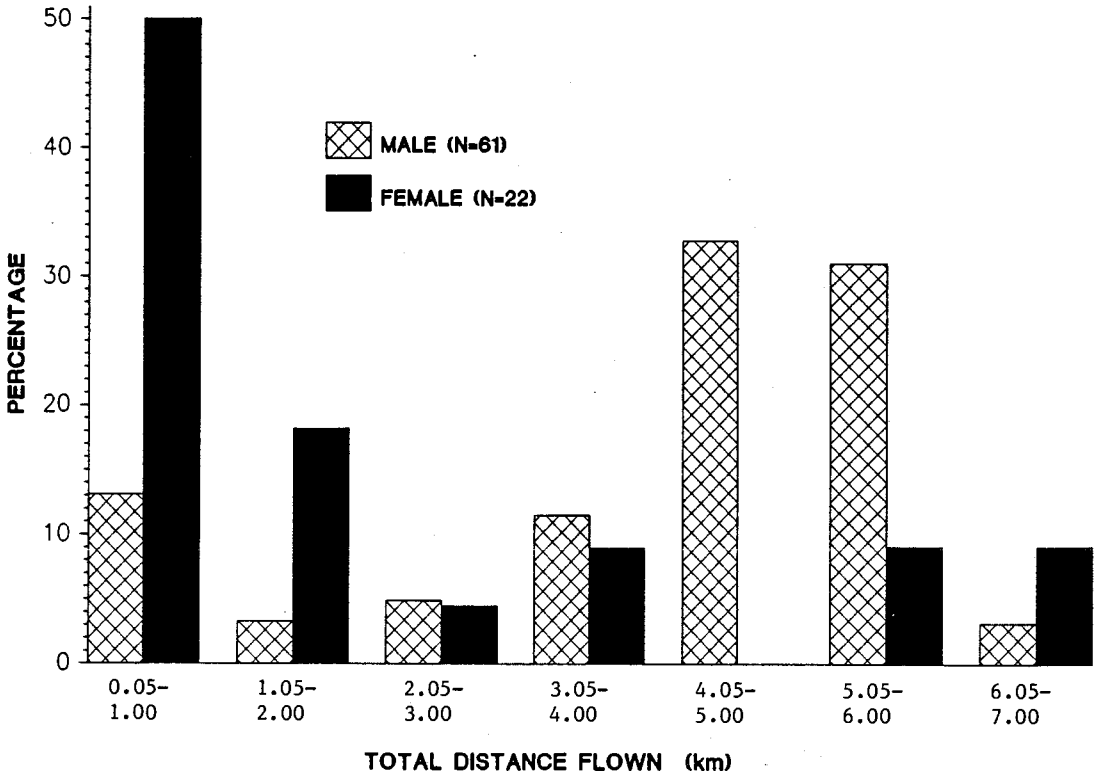
completed by 54.5 % of the moths. One individual flew continuously for the 5 h, travelling more than 20 km. Male OBLR averaged 5.7 flights (over 50 meters) in the 5 h test period; the number of flights per individual ranged between 0 and 22. Mean velocities for the longest flight completed by each moth averaged 1.01 m/sec (SE=0.032, N=64). Neither flight velocity nor total distance flown were significantly ($P > 0.05$) correlated with wing load or moth weight.

In contrast to the 5 h period used to examine male flight, female moths were tested for only 90 min. To facilitate a comparion between the flight abilities of male and female moths, female flight performance was compared with what males achieved in the first 90 min of their 5 h flight period. Note that total distance flown by male OBLR in 5 h was significantly ($P < 0.05$) correlated ($r= 0.713$) with distance flown in the initial 90 min.

Female OBLR flew significantly shorter distances than males (Mann-Whitney test, $P < 0.05$) (Fig. 7). The median distance for female moths was 1010 m, as compared to 4560 m for male moths. A comparison of flight velocities for male and female OBLR revealed no differences ($P > 0.05$). Mean velocity for the longest flights averaged 1.01 m/sec (SE=0.032, N=65) for male OBLR and 0.93 m/sec (SE=0.048, N=22) for females. A similar trend was observed for first flight velocities.

As observed for males, correlation analysis revealed that neither total distance flown nor flight velocity were significantly ($P > 0.05$) correlated with female weight or wingload. Comparison of size parameters between male and female moths revealed significant differences ($P < 0.05$). Female moths were on average, 3.55 times heavier, and had 1.62 times

Fig. 7. Total distance (km) flown by healthy male and female obliquebanded leafrollers in 90 min test period. This figure does not include 1 female and 2 male moths that failed to complete at least 1 flight longer than 50 m.


50
40
30
20
10
0
PERCENTAGE
MALE (N=61)
FEMALE (N=22)
0.05-
1.00
1.05-
2.00
2.05-
3.00
3.05-
4.00
4.05-
5.00
5.05-
6.00
6.05-
7.00
TOTAL DISTANCE FLOWN (km)

larger wing areas, resulting in 2.19 times greater wing loads than males.

4. Flight of healthy and N. fumiferanae-infected male C. rosaceana

Flight data for both healthy and infected moths were collected in 1989 and 1990. Preliminary analysis revealed sufficient differences between the 2 years to necessitate treating data from each of the years separately. Specifically, healthy moths flew significantly farther in 1989 than in 1990 (Mann-Whitney test, $P < 0.05$), and the wing areas of diseased moths were significantly (t-test, $P < 0.05$) smaller in 1989 than in 1990.

a) 1989 flight comparison

A comparison of wing areas, body weights and wing loads revealed that only wing areas were significantly ($P < 0.05$) different between healthy and diseased OBLR (Table 1). Wing areas for diseased moths averaged 7.8 % less than for healthy males.

N. fumiferanae spore concentrations, presented as a function of moth preflight weight, averaged 1.66×10^6 spores/mg (S.E.=2.29×10^5, N=21).

A comparison of distances flown during the first flight, longest flight and total 90 min test period revealed that first and total flight distances were significantly (Mann-Whitney test, $P < 0.05$) shorter for diseased moths (Table 2). For example, diseased moths flew a median total distance of 4035 m whereas healthy moths flew 5011 m. Subdivision of flight distances into two categories (Fig. 8), less than or greater than 1 km, disclosed no significant (X^2 test, $P > 0.05$) differences for any of the 3 flight distances measured . In other words, the proportion of flights in each of the distance categories were similar for diseased and

Table 1. Comparison of mean (± SE) body weights, wing areas and wing loads for healthy and *N. fumiferanae*-infected male OBLR flown in 1989.

TYPE OF MOTH	BODY WEIGHT[1] (mg)	WING AREA[1] (mm^2)	WING LOAD[1] (mg/mm^2)
HEALTHY	26.72 ± 0.56a (N=16)	263.91 ± 3.96a (N=16)	0.1012 ± 0.0012a (N=16)
INFECTED	25.11 ± 0.70a (N=21)	243.20 ± 3.51b (N=13)	0.1036 ± 0.0019a (N=13)

[1]Means in each column followed by different letters are significantly different (*t*-test, $P < 0.05$).

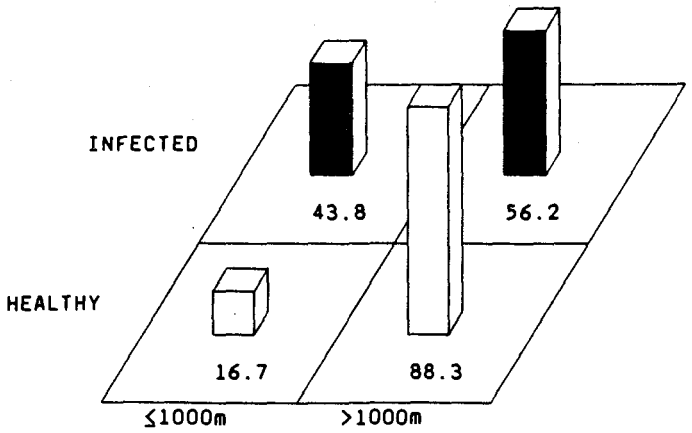
Table 2. Median distances (m) healthy and N. fumiferanae-infected male OBLR flew in 1989 and 1990.

YEAR	TYPE OF MOTH	TYPE OF FLIGHT		
		FIRST FLIGHT[1]	LONGEST FLIGHT[1]	TOTAL DISTANCE FLOWN[1]
1989	HEALTHY	4906a	4782c	5071d
	INFECTED	2272b	2547c	4035e
1990	HEALTHY	505a	1522b	3242c
	INFECTED	839a	1088b	2435c

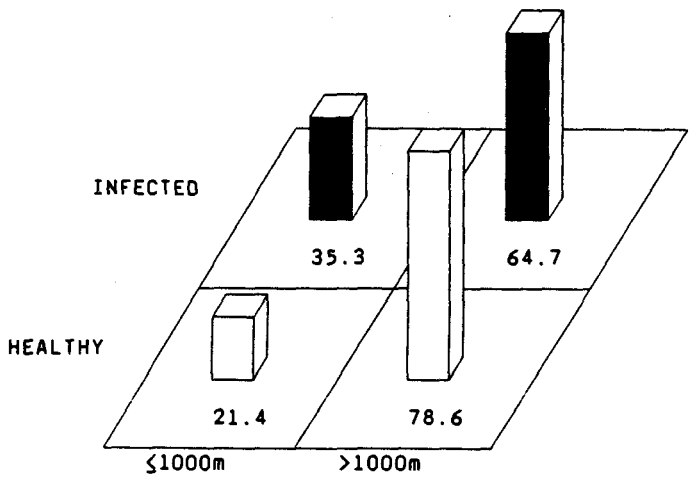
[1]Within a year, flight distances followed by different letters are significantly different (Mann-Whitney test, $P < 0.05$).

Fig. 8. Comparison of the percentages of healthy and N. fumiferanae -infected male OBLR in each of 2 flight distance (m) categories for 3 types of flight in 1989: a) First flight, b) Longest flight and c) Total distance flown. For all three flight types, the distribution of healthy and infected moth flight distances are not significantly different (X^2 test, $P > 0.05$).

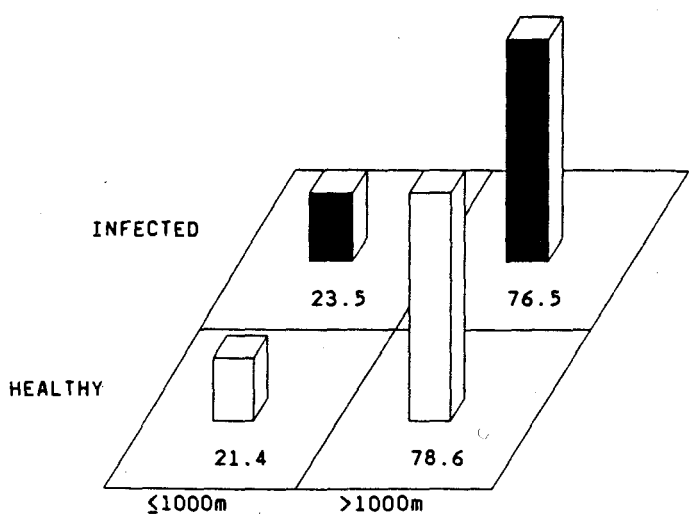
a)


INFECTED
43.8
56.2
HEALTHY
16.7
88.3
≤1000m
>1000m

b)


INFECTED
35.3
64.7
HEALTHY
21.4
78.6
≤1000m
>1000m

c)


INFECTED
23.5
76.5
HEALTHY
21.4
78.6
≤1000m
>1000m

healthy moths. A small and similar number of healthy and diseased moths failed to fly.

Analysis of flight velocities revealed more consistent differences. During both first and longest flights, diseased moths flew significantly ($P < 0.05$) slower than healthy moths (Table 3). For example, healthy moths averaged 0.94 m/sec during their longest flight while diseased moths averaged only 0.78 m/sec, a 17.0 % reduction. A similar trend was observed for first flight velocities.

An attempt was made to statistically compare the number of flights made by healthy and diseased moths. However, large variability and small sample sizes did not allow for a statistically meaningful analysis. That is, the large beta error would not allow me to accept the null hypothesis with confidence. Results suggest that diseased moths made more short flights than healthy moths. Diseased moths averaged 3.88 flights (range= 1-21) in the 90 min period while healthy moths averaged 1.36 flights (range= 1-4).

Finally, correlation analysis was used to evaluate the effect of N. fumiferanae spore load on diseased moth size and flight performance. None of the correlations were significant ($P > 0.05$).

b) 1990 flight comparison

No significant differences were detected between healthy and diseased moths with regard to weight, wing area or wing load (Table 4). Further, correlations of weight, wing area and wing load, as a function of N. fumiferanae concentration were not significant ($P > 0.05$). N. fumiferanae spore concentrations averaged 1.96×10^6 spores/mg ($SE=1.72 \times 10^5$,

Table 3. Mean velocities (m/sec) during longest flights for healthy and N. fumiferanae-infected male OBLR in 1989 and 1990.

YEAR	TYPE OF MOTH	MEAN VELOCITY[1] (± SE) (m/sec)
1989	HEALTHY	0.94 ± 0.057a
	INFECTED	0.78 ± 0.049b
1990	HEALTHY	0.85 ± 0.036a
	INFECTED	0.71 ± 0.028b

[1]Within a year, means followed by different letters are significantly different (t-test, $P < 0.05$).

Table 4. Comparison of mean (± SE) body weights, wing areas and wing loads for healthy and N. fumiferanae-infected male OBLR flown in 1990.

TYPE OF MOTH	BODY WEIGHT[1] (mg)	WING AREA[1] (mm^2)	WING LOAD[1] (mg/mm^2)
HEALTHY	27.03 ± 0.57a (N=21)	263.60 ± 3.78a (N=21)	0.1024 ± 0.0011a (N=21)
INFECTED	26.06 ± 0.48a (N=16)	258.82 ± 3.31a (N=16)	0.1001 ± 0.0017a (N=16)

[1]Means in each column followed by different letters are significantly different (t-test, $P < 0.05$).

N=16). This concentration is not different from that obtained in 1989.

In contrast to the 1989 results, no significant differences were detected between healthy and diseased moths for distances flown during first and longest flights, and total distance flown (Mann-Whitney test, $P > 0.05$) (Table 2). Examination of percentage of moths flying less than or greater than 1 km produced similar results. A similar proportion of healthy and diseased moths fell in each category (X^2 test, $P > 0.05$) (Fig. 9).

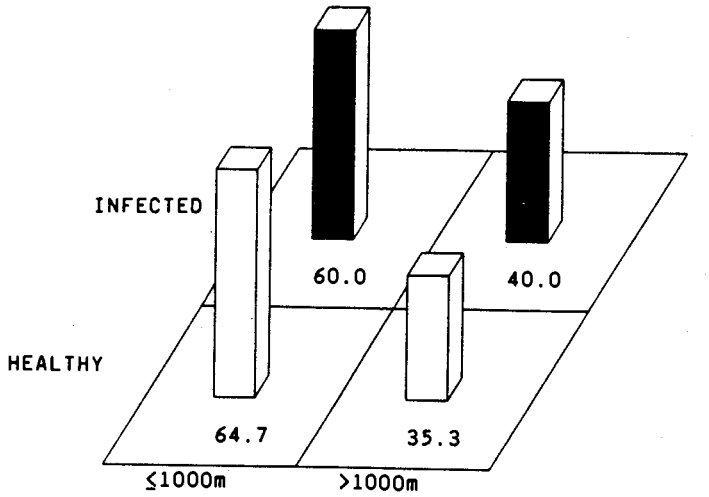
Average flight velocities for 1990 were similar to those obtained in 1989, with healthy moths flying significantly ($P < 0.05$) faster than diseased moths. For longest flights, healthy moths averaged 0.85 m/sec compared to 0.71 m/sec for diseased moths (Table 3).

As with the 1989 data, a meaningful statistical comparison of the number of flights by diseased and healthy moths was not possible. In contrast to 1989, healthy moths appeared to make more flights than diseased moths. Diseased moths averaged 1.88 flights (range= 1-6) while healthy moths averaged 2.84 (range= 1-11).

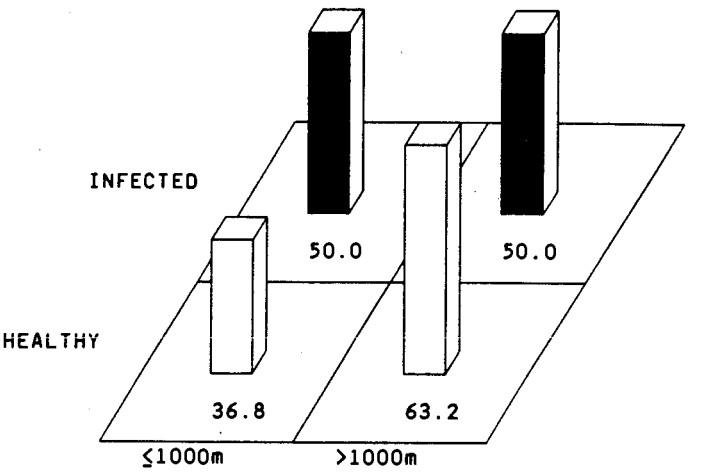
Correlation analysis revealed no significant ($P > 0.05$) influence of N. fumiferanae spore load on flight velocity or distances flown.

Fig. 9. Comparison of the percentages of healthy and N. fumiferanae -infected male OBLR in each of 2 flight distance (m) categories for 3 types of flight in 1990: a) First flight, b) Longest flight and c) Total distance flown. For all three flight types, the distribution of healthy and infected moth flight distances are not significantly different (X^2 test, $P > 0.05$).

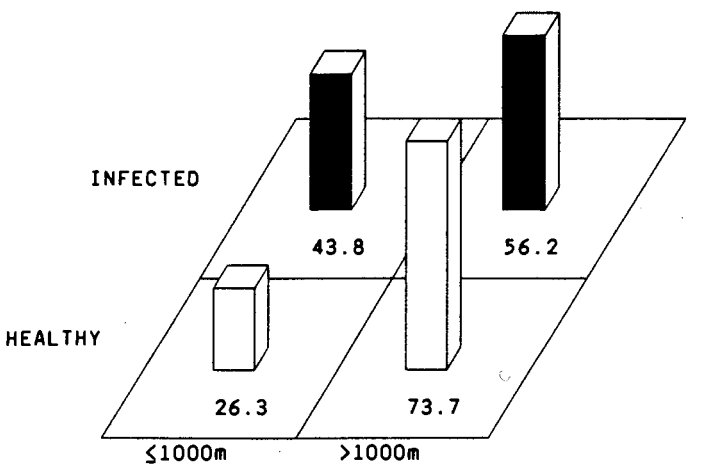
a)


INFECTED
HEALTHY
60.0
40.0
64.7
35.3
≤1000m
>1000m

b)


INFECTED
HEALTHY
50.0
50.0
36.8
63.2
≤1000m
>1000m

c)


INFECTED
HEALTHY
43.8
56.2
26.3
73.7
≤1000m
>1000m

V. DISCUSSION

Flight is essential for the survival of many insects. In particular, flight may be required for such activities as mating and dispersal. Any factor that interferes with the flight ability of an insect can potentially have serious detrimental effects on the dynamics of its population. This study assessed the flight ability of healthy male and female OBLR and then examined the effects of a N. fumiferanae infection on the flight performance of male OBLR.

Flight data acquired for healthy male and female OBLR indicated that the OBLR would readily perform on the laboratory flight mill system. The data also revealed that male OBLR were much more likely to fly long distances than were female OBLR (Fig. 6).

These data must be interpreted cautiously, particularly when speculating on the flight activity of OBLR in the field. While the flight mill system provides useful information on the potential physical flight ability of the moths, forced elimination of tarsal contact associated with attachment of a moth to the mill rotor causes the moth's normal flight decision making process to be by-passed. Therefore, field validation of any of the results obtained with the flight mill would substantially enhance the interpretation of data obtained with the flight mills. While no published field studies of OBLR flight exist, limited field observations do provide support for the results obtained with the flight mills.

Little is known about the flight activity of female OBLR. Limited field observations of young virgin females suggest that they move little, at least prior to mating (Y. Carrière, pers. comm.). Possibly,

females become more active fliers once they have produced a portion of their eggs and reduced their body weight.

Flight is unquestionably important to male OBLR. Male OBLR readily fly to pheromone-baited traps placed within orchards and other habitats occupied by OBLR. The daily sampling range, the maximum distance from which insects can reach a pheromone source in a day, is unknown for the OBLR. The pea moth, Cydia nigricana, a diurnal Tortricid, has been shown to have a daily sampling range of approximately 500 meters (Wall and Perry 1987). At least 1 male OBLR has been captured in a pheromone-baited trap, 2.5 km from the point of field release (J.E. Cossentine, pers. comm.). The moth completed the journey in 1 to 4 days. The dispersal activity of male OBLR has not been investigated.

Male OBLR were chosen for the evaluation of the effects of N. fumiferanae infection on OBLR flight because of their substantial flight activity. The preliminary longevity and histology studies demonstrated that N. fumiferanae had significant detrimental effects on male OBLR. A mean lifespan reduction of 48 percent for diseased moths, suggested that the pathogen utilized essential energy reserves and/or destroyed vital tissues. The histological study confirmed the presence of pathogen in tissues vital to flight. While only small clusters of spores were observed in thoracic muscle tissue, the cellular structure of fat body tissue was not discernible due to the extensive presence of N. fumiferanae. The very high N. fumiferanae spore concentrations found in diseased moths further suggested that they would lack the energy reserves required for prolonged flight.

The data from my study comparing the flight potential of healthy and N. fumiferanae-infected male OBLR, however, does not support the hypothesis that N. fumiferanae infection would substantially reduce the flight ability of male OBLR. Although in some instances, healthy moths flew significantly (Mann-Whitney test, $P < 0.05$) further than diseased moths (Table 2), N. fumiferanae-infected moths still flew considerable distances. Because the distances flown by both healthy and diseased moths were so great, the data were re-examined from a perspective that is likely more biologically meaningful. Assuming that male OBLR capable of flying 1 km could readily locate females (a conservative assumption?), re-evaluation of the data revealed that similar proportions of the healthy and N. fumiferanae-infected males flew sufficiently far to find mates (Fig. 8, Fig. 9) in the 90 min test period. Therefore, N. fumiferanae had considerably less influence on OBLR flight than expected.

My results are supported by data obtained for N. fumiferanae-infected eastern spruce budworm. Using a wind tunnel, Sanders and Wilson (1990) found that healthy and diseased male budworm spent similar time in flight tracking a pheromone plume. Their flight assessment averaged less than 5 min, however, while the present study evaluated flight over a 90 min period. The similarity of the results of the 2 studies is particular encouraging because, in the Sanders and Wilson (1990) study, the moths initiated flight naturally whereas in my study flight was initiated by elimination of tarsal contact when a moth was attached to a mill rotor. The willingness of diseased OBLR to initiate flight is further substantiated by my observations of diseased moths

flying in the 196 ml holding containers.

Mean flight velocities were consistently less for N. fumiferanae-infected OBLR than healthy male moths in both 1989 and 1990. Perhaps N. fumiferanae limited the rate of "fuel" availability at the cellular level. The effect of this 17 % reduction is difficult to assess. Possibly, because male OBLR follow pheromone plumes upwind, such a reduction in velocity would allow healthy moths to track pheromone plumes at higher wind velocities.

Generally, healthy and N. fumiferanae-infected male OBLR did not significantly differ in size (Table 1, Table 4). The one exception was in 1989, when diseased moths had smaller wings than their healthy counterparts. Average wing load, however, was not significantly different in either years. These results differ from those of many other studies where Nosema-infected adult moths often weighed significantly less than healthy moths. Perhaps, by exposing the OBLR to N. fumiferanae as mature larvae, the pathogen had little or no influence on the assimilation of food by the larvae.

Presumeably, flight performance would be influenced by the spore load carried by an insect. Unexpectedly, analysis did not reveal any correlation between spore concentration and either distance flown or flight velocity. The lack of observeable influence may be due to the limited range of spore concentrations observed within the N. fumiferanae-infected OBLR population.

Assuming that my results approximate those of a wild population, what do they suggest with regard to the dymanics of a host population, and what do they indicate regarding the use of N. fumiferanae for the

biocontrol of the OBLR?

My results suggest that the pathogen, N. fumiferanae, does not critically hinder the flight ability of male OBLR. While some reduction in flight performance was observed, diseased male OBLR should be physically capable of locating and mating with females. Of course, successful mating involves more than finding a female; the female must also be receptive. Although nothing is known about the acceptance of diseased male OBLR by conspecific females, N. fumiferanae-infected male eastern spruce budworm readily mated with females in laboratory experiments (Bauer and Nordin 1989). Just how competitive diseased male OBLR would be relative to healthy males, for available females, remains to be determined.

The effects of diseased male OBLR, that were mobile and readily mated with healthy females, on an OBLR population would depend on: 1) what effect, if any, diseased males had on the fecundity of healthy females, and 2) if diseased males could transmit the pathogen directly to their offspring. These issues are unresolved for the OBLR, and varying results have been reported for other host-pathogen relationships. If diseased male OBLR transmitted the pathogen vertically to their offspring, then dispersal of infected males would presumeably help to disperse the pathogen to healthy OBLR populations. This could be of particular significance for OBLR management because the OBLR is not restricted to orchards, but also occurs on a variety of native host plant species. Because N. fumiferanae is more likely transmitted vertically from infected females to their offspring, the flight activity of N. fumiferanae-infected females is perhaps more

important than the movement of diseased males.

VI. CONCLUSIONS

The results of this study suggest that the rotary flight mill system was suitable for comparing the flight ability of healthy and N. fumiferanae-infected OBLR. Some of my concerns about the use of flight mills, in particular the forced elimination of tarsal contact, would be addressed by a complementary study utilizing a wind tunnel. Although a wind tunnel would not allow for flight assessment over long time intervals, it would provide the moths with an opportunity to initiate flight naturally and to fly unrestrained. Assuming the results of a wind tunnel study substantiated my present results, the next step would be to assess the flight ability of N. fumiferanae-infected male OBLR in the field, by comparing the response of released healthy and diseased moths to pheromone-baited traps. Such a study could provide information on the distance travelled as well as the relative response of healthy and diseased OBLR. The results of a field study could also be used to assess the validity of the conclusions produced by the flight mill study.

However, at present N. fumiferanae has not been identified in wild OBLR in the Okanagan Valley and lack of knowledge about this host-pathogen association would make an introduction inappropriate at this time.

From a biocontrol perspective, it would be very useful to evaluate how N. fumiferanae effects the flight of female OBLR. Because of the potential competition between energy use for flight and egg production, no study of female flight would be complete without the simultaneous assessment of fecundity. Other important areas that

require examination include: how sublethal N. fumiferanae infection influences other adult OBLR life history traits, and the existence of both paternal and maternal vertical transmission of N. fumiferanae in the OBLR.

Ultimately, however, only controlled field releases of N. fumiferanae will provide a true asssessment of the influence of N. fumiferanae on wild OBLR populations.

VII. APPENDIX

PINTO BEAN-BASED DIET FOR OBLR

This recipe makes enough diet for approximately 400 - 28 ml cups.

(1) INGREDIENTS

a)	40.0 g	Agar
	720.0 ml	Water
b)	1600.0 ml	Water
	7.0 ml	Formaldehyde
c)	426.0 g	Pinto bean flour
	64.0 g	Brewers yeast
	6.4 g	Ascorbic acid
	4.0 g	Methyl-p-hydroxybenzoate
	2.0 g	Sorbic acid
	8.0 g	Vandervant™ vitamin mixture

(2) MIXING INSTRUCTIONS

a) Mix agar and 720 ml water. Bring to a boil and continue to heat until mixture thickens then remove heat.

b) Add additional water (1600 ml) and formaldehyde to agar mixture.

c) Add remaining ingredients, mix well, and dispense.

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