FEEDING STATUS OF COLORADO POTATO BEETLE (COLEOPTERA: CHRYSOMELIDAE): EFFECTS ON FLIGHT MUSCLE DEGENERATION AND FLIGHT ABILITY

by

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1992

ABSTRACT

FEEDING STATUS OF COLORADO POTATO BEETLE *LEPTINOTARSA DECEMLINEATA* (SAY) (COLEOPTERA: CHRYSOMELIDAE): EFFECTS ON FLIGHT MUSCLE DEGENERATION AND FLIGHT ABILILITY

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Flight tendency of starved and fed post-diapause and summer generation Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was measured in the field. There was no difference between the flight response of fed and starved beetles in either the post-diapause or summer generation experiments. In conjunction with the field flight experiment the ultrastructure of the dorso-longitudinal indirect flight muscles was measured in starved and fed post-diapause beetles. Ultrathin cross sections were examined using a Philips 201 transmission electron microscope. Degeneration of the fibrils and loss of cellular integrity began between 5 and 8 d of starvation. The muscle structure was maintained for fed beetles up to 15 d (end of sample period). Disorganization of cellular contents and reduced muscle fibrils were evident up to day 15 for starved beetles.

When the moon shall have faded out from the sky, and the sun shall shine at noonday a dull cherry-red, and the seas shall be frozen over, and the ice-cap shall have crept downward to the equator from either pole, and no keels shall cut the waters, nor wheels turn in mills, when all cities shall have long been dead and crumbled into dust, and all life shall be on the very last verge of extinction on this globe; then, on a bit of lichen, growing on the bald rocks beside the eternal snows of Panama, shall be seated a tiny insect, preening its antennae in the glow of the worn-out sun, representing the sole survival of animal life on this our earth,- a melancholy "bug".

-Author unknown

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr Ed Grafius for his support, and for allowing me the freedom to pursue my interests. I would also like to thank my committee members: Drs. Karen Klomparens, Jim Miller and George Ayers. I am very grateful for the expert advice and encouragement of Dr. Mary Rheuben, Department of Veterinary Medicine, M.S.U. The generous assistance, advice and great sense of humor of the staff in the Center for Electron Optics, M.S.U., was invaluable and much appreciated. I acknowledge the assistance of Heidi Tanis without whose help the field work would not have been possible.

Finally, Mark Wipfli- A notre avenir!

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Introduction

The potato, Solanum sp., was originally domesticated in the Andes of South America where it formed the staple of the native Indian diet. The arrival of the Spaniards in South America ensured the subsequent introduction of the potato into Europe. The potato has since been introduced into many parts of the world where it remains a staple food of the human diet and is a food source for some animals. Although wild Solanum spp. are found in the United States, the domesticated potato was brought to the U.S. from Ireland in 1719 by Scotch-Irish immigrants (Smith 1968). Potato production was initially centered in New England and the Middle Atlantic states. Potatoes subsequently were planted in the Midwestern states and eventually were grown in every state.

Potatoes are the most important root crop in terms of world production (Dalton 1978) and on the basis of total annual tonnage produced, the potato ranks fourth in the world after wheat, rice and maize (Harlan 1976). The potato supplies a nutritious carbohydrate food to about one-third of the world's total population (Hardenburg 1949). Easily processed or prepared, the potato is also a good nutritional source of protein, vitamin C and minerals. The potato is, however, most importantly consumed as a source of carbohydrates.

Potatoes are attacked by a variety of pests which damage tuber quality and reduce yields. Potato pests include fungi, weeds and insects, some of which came from the native potato habitats of South

America and others that have adapted to the cultivars grown in North America and Europe. Extensive management practices are employed to reduce the economic loss suffered from pest damage. Potato pests are controlled by cultural practices or agroecosystem manipulations, host resistance or genetic control, biological control, legal regulations and the use of pesticides (Thurston 1978). Many of these methods are in use in the Michigan potato growing regions in an effort to minimize damage by one of the most serious potato pests, the Colorado potato beetle, Leptinotarsa decemlineata (Say).

The Colorado potato beetle can cause extensive crop damage as a result of its voracious feeding habits. During a critical growth phase potato tuber production is reduced by as much as two-thirds as a result of 100% defoliation by Colorado potato beetle (Hare 1980). A native of Mexico, the Colorado Potato Beetle was first found in the United States along the eastern slopes of the Rocky Mountains (Radcliffe 1982). There it fed primarily on native Solanum species. As commercial potato production spread west the Colorado potato beetle readily shifted to S. tuberosum as its host. The Colorado potato beetle has since spread to most of the major potato growing regions of North America and Europe.

Adult Colorado potato beetles are 1-1.5 cm long, tan colored with 10 black longitudinal stripes on the elytra. The adult overwinters in the soil at a depth of 10-20 cm or sometimes deeper. The overwintered adult emerges from the ground as the soil warms in late May or early June (Hare 1990). Emergence will vary depending on temperature and location and beetles will emerge over an extended period (Lashomb et al. 1984). A range of temperatures from 8-10°C to 14°C has been reported for emergence from diapause and for post-diapause development (Wegorek 1959, Le Berre 1965, Lefevere and de Kort 1989, Tauber et al. 1988). Upon emergence, adults search for host plants and mates. This is done either by walking or by flight. In potato fields that are planted successive years with potatoes, walking may prove an effective means of dispersal in search of suitable host plants. However, in areas where potatoes are rotated with another crop, the beetle may emerge from overwintering faced with the need to disperse greater distances in search of food. Newly emerged post-diapause beetles will disperse by flight in the spring in search of food (Voss and Ferro 1990b and Caprio and Grafius 1990). Flight occurs at an optimum temperature of 20-25°C and typically does not occur below 15°C (Caprio and Grafius 1990). Le Berre (1950) determined that the duration of solar insolation is a major factor in flight initiation in the field. If food is immediately available the adults will feed and begin laying eggs. Grison (1947) indicated that the Colorado potato beetle must feed before laying eggs. Ferro et al. (1991), in a study of fed and unfed beetles, found that unfed females laid virtually no eggs. The eggs are bright orange and oval in shape and they are typically deposited on the underside of leaves in masses of 10-40 eggs. The eggs hatch in 7-10 days and the larvae go through 4 instars in 3-4 weeks. Mature larvae drop off of the plant and pupate in the soil; this stage lasts 10-15 days, whereupon a new generation emerges. Most populations in Michigan complete only 1 or 2 generations per year. However, in some areas of the United States there may be as many as three generation per year. In the fall, in response to a short-day photoperiod, beetles enter the ground to diapause. Beetles overwinter either in the field or they may migrate by flight to other overwintering sites (Voss & Ferro 1990a).

CPB Management

Devastating losses of potatoes to the Colorado Potato Beetle led to the use of one of the first insecticides, Paris Green, in the late 1800's (Gauthier et al. 1981). The use of this insecticide proved initially to be effective and precipitated the extensive use of insecticides for control of insect pests of many other crops. The development of insecticide resistance in the Colorado potato beetle has, however, rendered traditional insecticidal controls virtually ineffective in many locations. Many populations of Colorado potato beetle have developed resistance to nearly all currently registered insecticides (Hare 1990). Problems of insecticide resistance have necessitated implementation of alternate management practices. These include crop rotation, as well as the use of mechanical controls such as propane burners and vacuums. To maintain and improve management techniques it is necessary to further develop a detailed understanding of the biology of the Colorado potato beetle. Knowledge of the factors that influence or modify the behavior of this beetle could be used to manipulate this insect.

Dispersal is one such aspect of Colorado potato beetle biology that could be exploited for management purposes. A greater understanding of the dispersal capabilities of Colorado potato beetle, how they vary over the life-time and generations of the beetle and how some factors influence this dispersal ability could aid in optimizing alternate control practices.

Dispersal and Migration

There has been much debate in the insect literature over the definition of dispersal and migration. As long ago as the early 1900's authors have debated the definitions, distinctions and significance of movement by flight. Pearson and Blakeman, in 1906, postulated the existence of two kinds of flight: ‘The average distance through which an individual of the species moves from habitat to habitat will be spoken of as a "flight"... from locus of origin to breeding ground, or again from breeding ground to breeding ground, if the species reproduces more than once. A flight is to be distinguished from a “flitter”, a mere to and fro motion associated with the quest for food or mate in the neighborhood of the habitat’.

Since these early distinctions were made between the two different kinds of flight, numerous other authors studying the flight behavior of insects have grappled with the task of defining flight. Prominent authorities such as Southwood (1962) and Johnson (1969) have labelled the two separate flight behaviors as migratory flights and trivial or appetitive flights. Most authors tend to be in agreement with this distinction; it is, perhaps, more difficult to decide which behaviors of a particular insect are to be termed migratory and which trivial.

Trivial flight is usually considered to be flight that can be interrupted. That is, the flight is inhibited when another stimulus causes the insect to change its flight behavior and undertake a different behavior such as mating, oviposition, feeding etc. Matthews and Matthews (1978) defined trivial flight as involving 'local movements of varying length and orientation concerned with food and mate-finding, escape from potential enemies, location of suitable oviposition sites, territorial defense and other such "vegetative" activities'.

Johnson (1969), in his treatise on dispersal and migration, acknowledged that there has been some controversy about what migration is and how it differs from dispersal. He proposed that it is because the behavior of insects during dispersal and migration is both specific and variable that there has been so much disagreement among entomologists about the meaning of the word 'migration'.

Migratory movements typically take an insect away from one habitat or habitat type and into another habitat. Migration usually involves movement over long distances and migratory flight has inherent behavioral characteristics that are usually different from trivial flight.

It was previously thought that migration was a reaction to adverse conditions and that movement over long distances away from areas where, for example, the food supply had been depleted, into an area of abundant food ensured survival. This view has since been modified, with the recognition that insects did not always move away from adverse situations. Insects sometimes moved away from beneficial situations. It appears that the 'escape from' perspective may have a counterpart in an 'arrival at' perspective of insect migration. The insects' movements will necessarily take them to a new habitat that has the potential for colonization. This would be of benefit to insects in ensuring the spread and survival of genes.

Johnson (1960) pointed out that migratory movement generally occurs in the early part of an adult insect's life. The younger insects are typically not sexually mature when they undertake a migratory flight. As well, migration almost always involves the females of a species; however, not at the exclusion of their male counterparts. Southwood (1962) indicated that there are certain general behavioral characteristics of migratory flight. He reported that the take-off for flight usually follows a definite pattern in which the insect (in this case he refered mainly to aphids where much of the work on migration has been done) climbs to the top of the vegetation, faces the sun and flies upward. Taylor (1958) noted that this type of oriented behavior allows the insect to get up into the upper layers of the atmosphere where they are carried along by the wind.

These authors also notedthat many insects are positively phototactic and that they make relatively few and less frequent changes in direction than those insects engaging in trivial movement. Migratory insects are not usually responsive to the same stimuli as those insects that undertake appetitive flight. Migrating insects do not typically stop when they perceive a vegetative stimulus or a mate. Although certain insects that migrate very long distances, such as some Lepidoptera, may undergo a few generations during the course of their migratory route, from the place of origin to their "destination". As Southwood (1962) states, the thresholds for vegetative stimuli (mate, food etc.) are high during migratory movement and low during trivial movement.

Whatever the ecological or evolutionary need being satisfied by dispersal or migration there are inherent processes which provide the insect with the capability for movement. Involved in movement by flight are neurological, biochemical and morphological/physiological processes. These processes are intimately linked to allow the whole insect to function. The function of movement and flight is influenced to differing degrees by various factors.

Understanding the mechanisms of movement in insect pests is a complex procedure. One approach is to isolate the various components of flight to determine the specific factors that may influence this capability for flight. Skinner et al. (1983) suggested four areas in which research is critically needed to understand the role of movement in elucidating the dynamics of pest species. One of these suggested areas is the identification of physiological and behavioral mechanisms conducive to initiation, orientation, maintenance and termination of flight.

The insect flight muscle system is one such physiological/morphological component whose utilization produces a direct behavior response, flight dispersal, in the insect. Mature flight muscles constitute one of the necessary elements in an insect's development that makes it capable of flight.

Flight muscles

The flight musculature is contained within the meso and meta thoracic segments of the insect. In most insects the flight musculature comprises five principal sets of muscles: the dorsal muscles, the tergo-sternal muscles, the axillary muscles, the basalar muscles and the subalar muscles (Snodgrass 1935). These sets of muscles work to produce the movements of the wing. The wings of an insect are complicated in action, involving the up and down stroke, the fore and aft movements and twisting movements.

The dorso-longitudinal and tergosternal muscles are referred to as the indirect flight muscles and, in Coleoptera, act to change the shape of the thoracic skeleton during contraction (and relaxation). The dorso-longitudinal muscles are composed of the median longitudinal and the lateral oblique muscles. Contraction of the dorso-longitudinal muscles causes an arch in the wing-bearing terga resulting in depression of the wings. The antagonistic contraction of the tergosternal muscles flattens the terga causing the upstroke of the wings. The basalar and subalar muscles act in part to rotate the wing forward and backward with the up and down stroke. The subalar muscle also contributes to the depression or down-stroke, in conjunction with the dorso-longitudinal muscles. The subalar and basalar are also considered to be indirect flight muscles. The axillary muscles are the only muscles attached directly to the base of the wing and are termed direct flight muscles. They are responsible for the curvature or twisting of the wing during flight. The whole movement of the wing, therefore, traces with its leading edge the form of a figure eight during flight.

There are other intersegmental muscles that, while not principal contributors to the movement of the wing, have some relation to the movements of the wing by influencing the movement of the tergum (Snodgrass 1935). The component muscles of this generalized flight musculature can vary somewhat amongst Orders. The extent of muscle development can also vary, being practically non-existent in some insects to fully developed in others. The Colorado potato beetle has well developed pterothoracic muscles.

The flight muscles of insects are striated, with the Z-bands lined up in a relatively organized fashion. Coleoptera, along with other higher Orders, have fibrillar flight muscles. Fibrillar muscle are characterized by big fibers and fibrils with large sarcosomes occupying the spaces between the fibrils. These muscles are well tracheated providing a rich supply of oxygen. They are also characterized by a high wing beat frequency, unlike the wing beat frequency associated with non-fibrillar flight muscles. In fibrillar flight muscles the contraction rate is not related to action potential frequency; neural stimulation causes an oscillation in the contractile units of the muscle.

From this generalized schema of flight muscle structure there can be much variation among insect species. Johnson (1969) observed that within a population of a single species some, or all, of the individuals may never develop all of the necessary structures or the ability to fly. After developing the faculty and using it in flights, they may subsequently lose the associated structures and hence the ability to fly. Henson (1961) reported that in both sexes of Cnophthorus coniperda (Shwartz) the dorso-longitudinal and tergo-sternal muscles shrink to thin threads while other muscles do not degenerate. In this insect the degeneration process occurs suddenly, in June, after the adults have come out of hibernation; however, in some individuals muscles regenerate and are present in July, but a second period of degeneration begins in August and coincides with senescence.

This phenomenon of muscle development, degeneration and regeneration can be influenced by various external and internal factors. The physiological status of the insect has a direct influence on the muscle ultrastructure, especially during metamorphosis. Food, in some insects, has been shown to play a role in flight muscle development and flight behavior. In some species of Corixidae, full development of the flight muscles depends on adequate food intake during the teneral stage. A shortage of food retards or even prevents development of flight muscles and the amount of food partly determines whether those insects develop into flying or flightless forms (Young 1965).

A similar process of muscle growth occurs in tsetse flies (Glossinia sp.); although, these insects must fly first in order to feed before the muscles are fully developed (Bursell 1961). Several species of insects are known to have a polymorphism involving the flight muscles but the effect of food on their development has not been extensively studied.

Colorado Potato Beetle Flight

Much work has been done on flight initiation in Colorado potato beetle; however, comparatively little work has been done on the development of the flight muscle system and its relation to flight behavior. Le Berre (1966) investigated the flight behavior of adult beetles in France and determined that Colorado potato beetle flight ability is not constant throughout its life. He reported that the beetle has a period where it is unable to fly, then develops an increasing ability to fly which will eventually decline with age. Under infra-red lights with beetles being free to move at will, Le Berre (1952) found that fed beetles flew more readily than unfed beetles. Unfed beetles did not initiate flight until the 8th day of testing compared to the fed beetles which initiated flight on the 3rd day of testing. Other observations made by Le Berre (1950) indicated that the spring generation of beetles can fly without being fed; whereas, the summer generation needs to feed heavily for the first 10 days post-emergence for flight to occur. Caprio and Grafius (1990), on the other hand, noted a tendency for starved beetles to fly more frequently than fed beetles after overwintering. Voss and Ferro (1990b) report two periods of flight activity in the Colorado potato beetle: the first by the post-diapause adults and the second by the first generation summer adults.

Brouwers and de Kort (1979), in their examination of the energy substrate for flight in long-day versus short-day summer adult Colorado potato beetles, observed that flight was greatest with 7-15 day old beetles. In a comparison of long-day and short-day beetles Mordue and de Kort (1978) found that short-day beetles could not be induced to fly; whereas, long-day reproducing females could be induced to fly for short periods (3 min). However, beetles newly emerged from diapause (mainly females 4-8 days post-diapause and undergoing maximum reproductive development) flew for up to one hour. These authors found that few of the post-diapause males flew.

As mentioned for other insects, flight muscle development may be one of the factors that cause differential flight behaviors or abilities in the Colorado potato beetle. de Kort (1969) observed a gradation in the development of flight muscles (mostly males were used in this study) in the Colorado potato beetle. A significant development of the flight muscles takes place after emergence from pupation and the growth of the fibrils is complete after about 5 days, with maximum development occurring at about 9 days. He noted that the increase in flight muscle size up to 5 days roughly coincides with the attainment of flight ability that Le Berre (1966) reported to occur at 4 days of age. Stegwee et al. (1963) studied the muscle structure of diapausing potato beetles and showed that muscle degeneration occurs during diapause. Regeneration of the indirect flight muscles occurs shortly before termination of diapause. The post-diapause generation, therefore, emerges with fully developed flight muscles making it potentially capable of efficient dispersal, but also leaving oportunities for manipulation.

Combining the observations made in these studies, it appears that there may be a link between the development of the indirect flight muscles and the ability of the Colorado potato beetle to fly.

Stegwee's studies show that the Colorado potato beetle has the potential to elicit degeneration and regeneration of the indirect flight muscles in response to environmental conditions and physiological demands during diapause. If this differential in muscle development and corresponding changes in flight ability occur in response to feeding regimes with the dispersal prone generations (i.e. the overwintered and the first generation summer adults) there are obvious implications for management of these populations.

Stegwee and de Kort showed that under natural growth conditions of proper food, light and temperature, changes occur in the indirect flight muscles of the Colorado potato beetle during certain stages of adult life. However, the relationship between the effect of food on the ultrastructure of flight muscles and the effect of flight muscle changes on the ability of the insect to fly has not been investigated. An understanding of this relationship is important in determining the conditions that may affect dispersal capabilities of post-diapause Colorado potato beetles. This knowledge would aid in the effective management of pest populations through manipulation of the host plant-pest environment.

The objective of this thesis was1) to determine the flight response of fed and starved post-diapause and summer generation Colorado potato beetles in a field situation, and 2) to elucidate and compare the ultrastructural status of the dorso-longitudinal indirect flight muscles in fed and starved post-diapause Colorado potato beetles. Ultimately the purpose of the combined studies, conducted simultaneously, was to provide a basis for a structure/function correlation between the flight response of starved and fed post-diapause beetles and the flight muscle ultrastructure of the same beetles. This information will be useful in designing management strategies for control of this serious pest.

Chapter 1

The effect of starvation on flight behavior of post-diapause and summer generation Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

Introduction

Although the Colorado potato beetle, Leptinotarsa decemlineata (Say), is not a strong flier, it is known to disperse by flight. The Colorado potato beetle flies at different periods in its adult life: Tower (1906), in North America, and de Wilde and Hsaio (1981), in France, report that more flight occurred in the late summer and early fall. Voss and Ferro (1990b), in Western Massachusetts, observed flight activity by post-diapause beetles in the spring and by the first of two summer generations in mid-summer. They observed three distinct types of flight: local flight in and around the study field, long distance flight out of the field and pre-diapause flight to nearby woods.

Spring flight enables post-diapause beetles to locate host plants. The beetle engages in either a short flight within or to nearby potato fields or in a longer flight in search of available food. When the beetle emerges before the potato plants or not near food it may undergo a period of starvation. Starved and fed beetles do not necessarily exhibit the same flight behavior (LeBerre 1966, Caprio & Grafius 1990, and Ferro et al. 1991). LeBerre notes that when post-diapause beetles are starved there is a rapid decrease in the flight ability of both sexes. He attributed this inability to fly to a decrease in the beetles' energy reserves and to a weakening of the flight muscles. After re-establishing a feeding regime, starved beetles regained their ability to fly within 5 days. A field study by Caprio & Grafius (1990) showed that beetles were capable of dispersing in the spring without prior feeding and they suggested that beetles may disperse over long distances at this time. They observed two separate flight behaviors: short, low flights by fed beetles that terminated on host plants, and longer (>100m), downwind flights, at a greater height, exhibited by starved beetles. In a laboratory flight mill study, Ferro et al. (1991) showed that unfed beetles flew more often, for longer periods and for greater distances than fed beetles; unfed beetles also flew more in the first 15 days than did fed beetles. They also noted that first flights for unfed beetles occurred sooner than for fed beetles.

The objective of the present study was to determine whether, in a field situation, starvation and the length of starvation affect the flight behavior of starved Colorado potato beetles compared to fed beetles. I examined the flight response (whether beetles flew and how far), over time, of post-diapause beetles and of the first-generation summer adults.

Materials and Methods

Post-diapause experiments. Overwintered adult Colorado potato beetles were collected at the Michigan State University Montcalm Potato Research Farm (Montcalm Co., MI) on May 16th and

20th, 1991. I collected newly emerged beetles from the soil surface or from small potato plants (<5 cm high). The beetles that were collected from plants had not fed extensively, as a relatively small portion of the leaves was eaten and most beetles were not actively eating when collected.

In the lab the beetles were placed into covered plastic containers (12 cm diameter X 8.5 cm high) that were filled to within 5 cm of the top with moist potting soil. Small holes were poked in the sides and top of the plastic pots. The pots were placed in a growth chamber set at 10°C with a 12:12 photoperiod. Containers were stacked in the chamber so that each container was assured appropriate photoperiod exposure. I conducted three experiments to observe the flight behavior (whether the beetles flew) of these overwintered beetles.

For experiments 1 and 2 I used beetles collected on May 20, from small potato plants. A subset of these beetles was removed from the growth chamber on June 13, 1991 for experiment 1 and on June 25 for experiment 2. A subset of beetles from those collected on May 16 from the soil surface was removed from the growth chamber on July 9 for experiment 3. Each beetle was sexed and randomly assigned to one of four replicates for either of two treatments: 1) Fed (control), 2) Starved. Each replicate contained 30 beetles. Each beetle was coded with a dot of colored paint applied to the prothorax to identify the sex of the beetle, the treatment and the replicate. For the fed control, each replicate consisted of one potted Superior var. potato plant enclosed in a screen mesh cage. The potato plants were replaced as needed. For the starved treatment, each replicate consisted of a soil-filled pot, identical to the control pot, containing a petri-dish with wet filter paper. All replicates were placed in a controlled room at 27°C and an 18:6 L:D photoperiod. The duration of the test period varied for each experiment. Flight behavior was examined on day 1,2,3,4,5,6,8,11,15,19 after removal from the growth chamber, for experiment 1; day 1,2,3,4,5,6,8,10,15,21,28 for experiment 2; and day 1,2,3,4,5,6,8,15,17,19,22,24,27,30,35 for experiment 3. On each test day one beetle was randomly selected from each of the replicates (four beetles/treatment; 2 female, 2 male) and tested for flight behavior. The beetles subjected to the flight behavior testing were not returned to the treatments. Flight behavior experiments were conducted at the Michigan State University Entomology Field Research Farm (Ingham Co. MI). All beetles were placed on a bare soil surface within a 27.5 cm square plexi-glass barrier (8 cm high) coated along the top 2.5 cm with Fluon™ to prevent the beetles climbing the plexi-glass. Five sticks (17.5 cm high, 1 cm diameter) were placed on end in the container for beetles to climb on (less than half of the beetles used the sticks). Tests were conducted at midday and the beetles were observed for one hour. Flight (defined as exceeding the boundaries of the container) was recorded for each beetle.

Summer Generation Experiment. Mature 4th instars were collected from potato fields in Lapeer Co., MI on August 6, 1991. The beetles were fed in the lab on potato plants (c.v. Superior) until they burrowed into the soil for pupation. The foliage was removed and pupating beetles were maintained at 25°C, 18:6 L:D photoperiod. Upon emergence the adult beetles were collected and placed on Superior var. potato plants. Beetles emerged over a 3 d period and were, therefore, allowed to feed for 5-8 d. I then combined the beetles and randomly assigned beetles to one of 20 replicates (8-9 beetles/replicate) for each of two treatments: 1) Fed-control, or 2) Starved. Beetles for this experiment were not sexed. Each replicate was either fed or starved as stated for the post-diapause experiments.

Flight behavior was tested in the field at the Entomology Farm 1,4,10 & 14 days after the beetles had been assigned to a treatment. For each flight test 4 replicates were picked at random from each treatment and the number of beetles present in that replicate was recorded (some beetles died and others went into a diapause state at the end of the experiment). Beetles were discarded after each test day. Flight tests were conducted as previously described except that I used two plexi-glass containers because more beetles were being tested. The estimated distance of each beetle's flight was recorded.

Data was analyzed by ANOVA (p=.05).

Results and Discussion

Post-Diapause Experiments. In experiment 1, no starved beetles flew after 6 days (Figure 1). Those that did fly, flew after 2,3,4 and 5 days of starvation. I saw flight for the fed beetles on day 2,3,4,8, and 11 for the fed beetles. There was no significant difference between the numbers of starved and fed beetles that flew; although the trend in Experiment 1 and 2 indicated that more starved beetles flew compared to fed beetles.. Experiment 1 was conducted during the latter half of June when the temperature exceeded 20°C. Caprio and Grafius (1990) found that at 20°C all starved post-diapause beetles flew. Their beetles had been starved 1-2 weeks which was comparable to my experiment.

In experiment 2 (June 26-July 23), I saw generally very little flight activity (Figure 2). Flight that occurred was after 2,3,8,10 and 15 days of starvation and I saw minimal flight after 3 and 21 days for the fed beetles.

I saw more flight by both starved and fed beetles in experiment 3 (July 10 and August 12) than in experiments 1 or 2 (Figure 3). There was virtually no flight during the first six days of starvation. It was only after 10 days of starvation that the beetles started to fly and flight by both groups was seen up to 27 days of treatment. These results differ from both Ferro et al. (1991), who found that unfed beetles, tethered to a flight mill in the laboratory, flew more often than fed beetles, and Caprio and Grafius (1990) who found, in field experiments, that starved beetles flew more than fed beetles.

The results of the three experiments using post-diapause beetles showed that there was no significant difference ($p>0.05$) between the number of starved and fed Colorado potato beetles that flew. There was also no significant effect of the length of the starvation time on the flight activity ($p>0.05$).

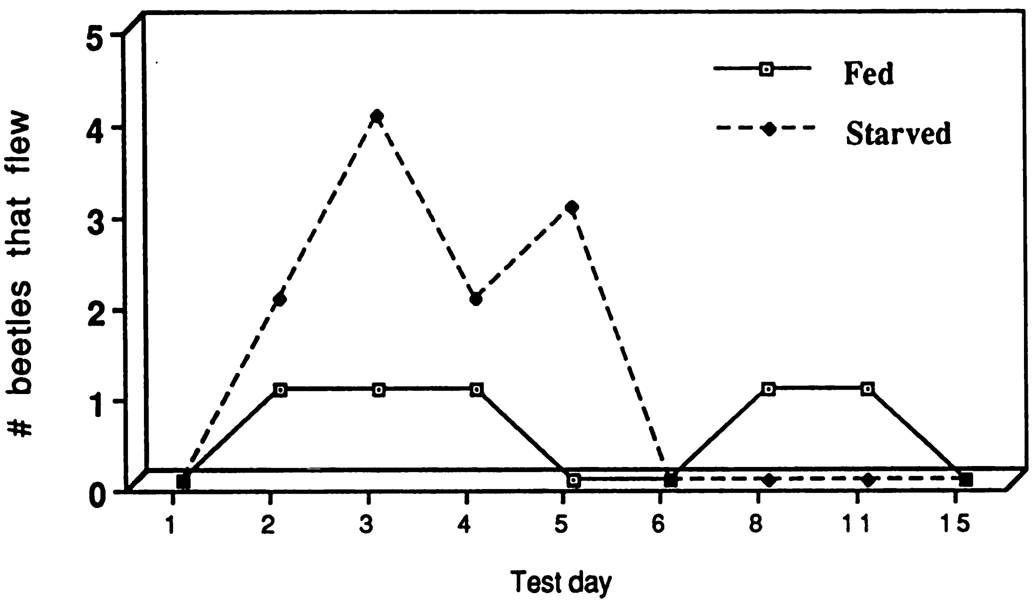

Figure 1. Experiment 1. Total number of post-diapause Colorado potato beetles/treatment that flew (flight defined as exceeding the boundaries of the test arena) on each test day.

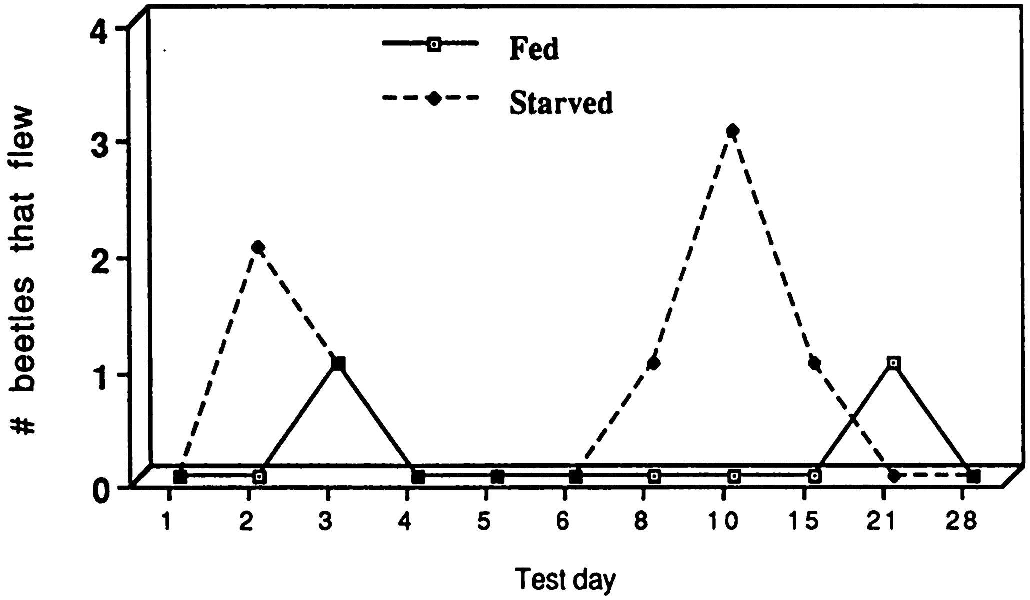

Figure 2. Experiment 2. Total number of post-diapause Colorado potato beetles/treatment that flew (flight defined as exceeding the boundaries of the test arena) on each test day.

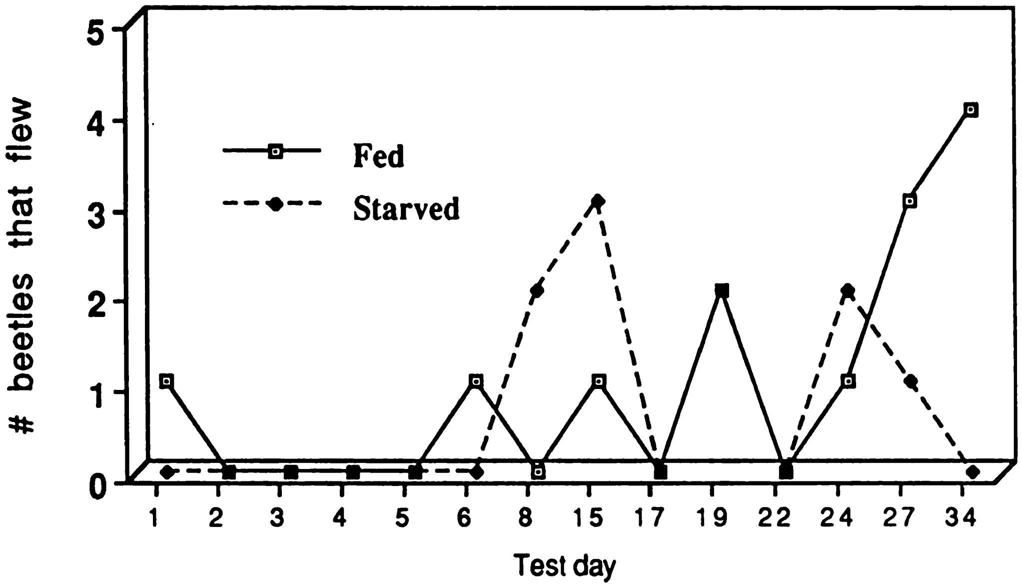

Figure 3. Experiment 3. Total number of post-diapause Colorado potato beetles/treatment that flew (flight defined as exceeding the boundaries of the test arena) on each test day.

Flight by all post-diapause beetles was typically <2m high and ranged from <1m in distance to >90m (beetles were out of sight). The height and distances of these flights indicate that these could be food-searching flights. The weather conditions did not appear to influence the flight behavior. The beetles flew on days when it was sunny, cloudy or with overcast dark clouds; I also recorded no flight under similar weather conditions. Solar insolation, which Le Berre (1950) considered an important factor to flight initiation in Colorado potato beetle, did not appear to be a limiting factor in my study. The temperatures in my study were never below 20°C and were usually 30-35°C. Caprio and Grafius (1990) found that all starved beetles flew at 20°C but it has not, to my knowledge, been determined whether there is an upper range that might influence the beetles' flight ability or flight initiation. However, Grafius (1986) reported reduced beetles feeding at 35°C and rapid mortality at 40°C. Perhaps some of the temperatures that my beetles encountered on particularily hot days were inhibitory. On very hot days beetles did seem to seek the shade of the test barrier.

Summer Generation Experiment. The flight behavior test (August 27 to September 9) showed no significant difference ($p>0.05$) between the percent flight of starved and fed beetles (Figure 4). There was, however, a significant date effect ($p<0.001$, $F=12.7$). Ninety percent of the starved and 72% of the fed beetles flew after 4 days of treatment. The percent flight of both fed and starved beetles for the other three test days (1,10 and 14 days of treatment) ranged from 20% and 30% (respectively) on test day 1 to 39% and 55% on test day 10 and 23% and 17% of test day 14. The block (date) effect could be due to a number of factors: temperature, weather conditions, wind (probably would not have greatly affected the flight intiation because the beetles on the ground were shielded from the wind by the test barrier), amount of solar insolation or age of the beetles. As in the previous experiments there does not appear to be a trend towards increasing solar insolation promoting more flight initiation. Although solar insolation was not directly measured, the flight was generally less on day 1, when it was sunny, than on day 10, when it was partly cloudy. On day 14, when it was also partly cloudy, I recorded a similar percent flight to day 10. None of these differences was, however, significant. The date effect could likely have been due to a combination of factors.

It appears, also, that the number of beetles that flew in each of my established categories (1=no flight; 2=<30m; 3=30-90m; 4=>90m) (Fig.5-8) did not differ consistently among categories. The beetles tended to fly <2m high and I recorded distances of up to 90m. Some beetles flew greater distances but I could not follow them to determine where they landed.

My study showed that for both post-diapause and summer Colorado potato beetles the starved and fed beetles did not exhibit different flight behavior. The length of starvation or feeding did not affect the flight tendancy of beetles in the post-diapause generation but there was an effect of treatment time (date) on the summer generation which I could not attribute to a single factor.

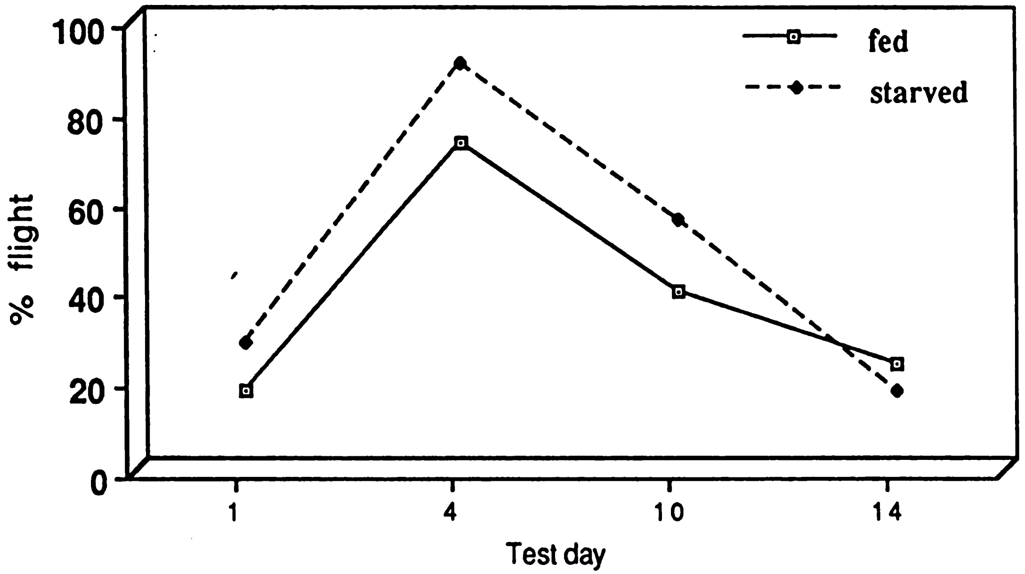

Figure 4. Percentage of starved and fed summer generation Colorado potato beetles that flew (flight defined as exceeding the boundaries of the test arena).

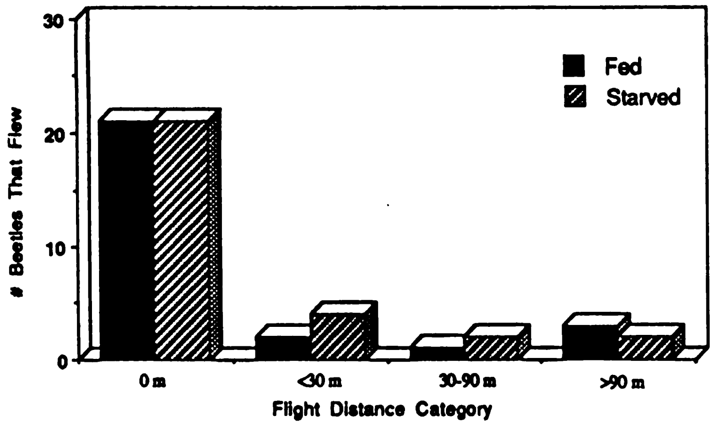

Figure 5. August 27, 1991. Number of summer generation Colorado potato beetles that flew (out of the test arena) in each of four categories.

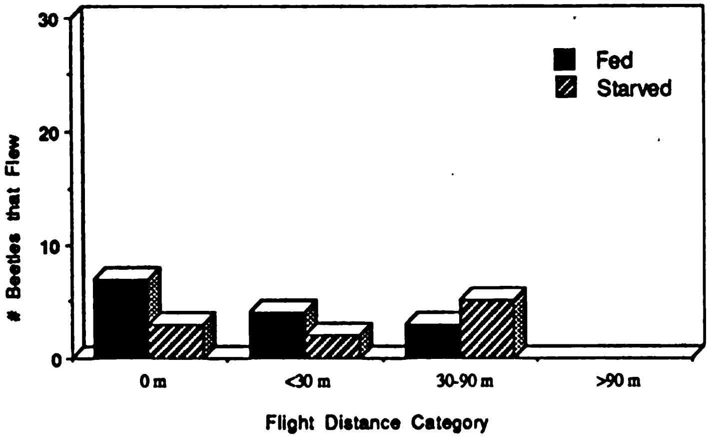

Figure 6. August 30, 1991. Number of summer generation Colorado potato beetles that flew (out of the test arena) in each of four categories.

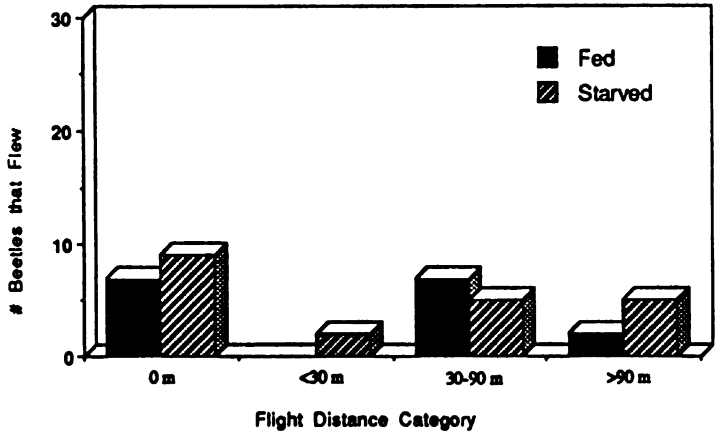

Figure 7. September 5, 1991. Number of summer genereation Colorado potato beetles that flew (out of the test arena) in each of four categories.

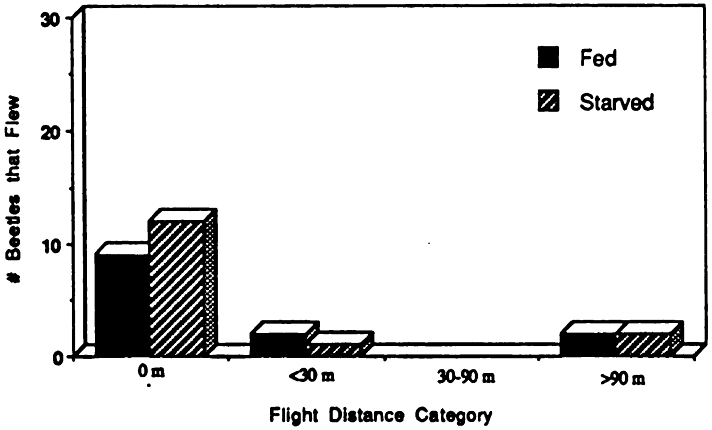

Figure 8. September 9, 1991. Number of summer generation Colorado potato beetles that flew (out of the test arena) in each of four categories.

Chapter 2

Ultrastructural analysis of dorso-longitudinal indirect flight muscles in starved and fed post-diapause Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

Introduction

The musculature of insects goes through a natural process of change as holometabolous insects pass through the different life stages from larva to adult. The larval muscles degenerate and the functional adult muscles are formed. Once the insect reaches the adult stage, however, some muscles also have the potential to degenerate and regenerate. The process of muscle degeneration has been studied in detail during metamorphosis of holometabolous insects (Auber-Thomay 1967; Crossley 1968; Randall and Pipa 1969; Lockshin and Beaulaton 1974; Lockshin 1975; Beaulaton and Lockshin 1977; Rheuben, in press). Experimentally-induced degeneration in insect muscle has also been studied in association with denervation (Rees and Usherwood 1972), tenotomy (Jahromi and Bloom 1979) and application of juvenile hormone analog to adult flight muscle (Unnithan and Nair 1977). Most of the studies of flight muscle degeneration have examined the natural occurrence of this phenomenon. One focus of insect muscle degeneration has been the indirect flight muscles of adult insects. In several insect species, degeneration of adult flight muscle is associated with growth of ovaries, egg-production and insemination in females. This phenomenon was documented in several scolytid beetles, (Chapman

1956; Reid 1958; and Atkins & Farris 1962) and in the ant, Solenopsis, (Jones, Davis and Vinson 1982).

Atrophy of indirect flight muscles has also been reported for some Diptera (Hocking 1954 and Mercier 1924). For some species of mosquito, atrophy is associated with mature eggs while in other species there is no association. Flight muscle degeneration after settling has also been reported in several species of aphids (Johnson, 1957, 1959, 1980) and muscle degeneration after a single flight has been reported in the queens of an ant and a termite species (Janet 1907, and Feytaud 1912).

Smith (1964) noted muscle atrophy in the fibrillar flight muscles of four species of Coleoptera. These studies included different groups of flight muscles; however, a similar overall process of degeneration occured. Degeneration usually involved a decrease in fibril diameter, crowding of nuclei and a general disorganization of the cell. The process of degeneration (as noted by Smith, 1964) did not occur uniformly or consistently in all muscles studied nor did it occur at the same stages in adult life. It was not clear, in these instances why this degeneration occured.

In some insects, muscle degeneration is followed at some point by regeneration. For some scolytids this is seen in response to repeated attacks on trees (Reid 1958; Chapman 1956; Chapman & Kinghorn 1958; Bhakthan, Borden and Nair 1970). In other insects, this process of histolysis occurs to coincide with diapause and the emergence from diapause (Ryan 1959, Ebbe-Nyman 1952).

In Coleoptera, there are two sets of large indirect flight muscles, along with some smaller muscles that contribute the power for flight.

The tergo-sternal and the dorso-longitudinal muscles work antagonistically to produce much of the up and down motion of the insect wing (Snodgrass 1935). Stegwee et al. (1963) reported that Colorado potato beetles Leptinotarsa decemlineata (Say) degenerate their tergo-sternal indirect flight muscles after they enter diapause and regenerate these muscle shortly before emergence in the spring. A study by de Kort (1969) examined the development of the dorso-longitudinal flight muscles in the post-pupal adult Colorado potato beetle. These two studies are the extent of the existing literature on muscle ultrastructure in Colorado potato beetle. In this study I chose to concentrate on one of these groups of muscles- the dorso-longitudinal muscles.

Colorado potato beetle is an economically important pest of potatoes in much of the United States and Eastern Canada. Since it emerges from diapause with fully developed muscles the potential for dispersal is great. Colorado potato beetle can, however, emerge before any food source is available or may emerge in locations where there is no suitable food, inflicting a potential period of starvation. Starvation and the relationship to flight muscle status could have implications for the control and management of this pest.

The objective of this study was to determine the effect of feeding and starvation, as well as the length of starvation, on the ultrastructure of the dorso-longitudinal indirect flight muscle of the Colorado potato beetle. I documented the ultrastructural changes and their relarionship to the length of starvation time.

Methods and Materials

Treatment set. Overwintered adult Colorado potato beetles were collected at the Michigan State University Montcalm Potato Research farm (Montcalm Co., MI) on May 16th and 20th 1991. Beetles had just started to emerge from the soil when they were collected either from the soil surface or from small potato plants (<5 cm high). The beetles that were collected from plants had not fed extensively as a relatively small portion of the leaves was eaten and most beetles were not actively eating when collected.

In the lab, the beetles were placed into plastic pots (12 cm diameter x 8.5 cm high) that were filled to within 2.5 cm of the top with moistened potting soil. Small holes were made in the sides and top of the plastic pots. The pots were placed in a growth chamber set at 11°C with a 12:12 L:D photoperiod.

I conducted three tests with these post-diapause beetles to observe the flight behavior of fed and starved beetles (Chapter 1). The same beetles used for the flight behavior experiments were dissected and examined to determine the status of the dorso-longitudinal indirect flight muscles after exposure to varying periods of starvation.

For tests 1 and 2, I used beetles collected on May 20 from small potato plants. A subset of these beetles was removed from the growth chamber on June 13 for test 1 and on June 25 for test 2. A subset of beetles from those collected on May 16 from the soil surface was removed from the growth chamber on July 9 for test 3. For each trial, beetles were sexed and randomly assigned to one of two treatments: 1) Fed (control) and 2) Starved, four replicates per treatment, 30 beetles per replication. Each beetle was color coded with a dot of paint applied to the prothorax to identify the sex of the beetle, the treatment and the replicate. For the fed control, each replicate consisted of one potted potato plant (c.v. Superior) enclosed in a screen mesh cage. The potato plants were replaced as needed. For the starved treatment, each replicate consisted of a soil-filled pot, identical to the control pot, containing a petri-dish with wet filter paper. All replicates were placed in a controlled room at 27°C with an 18:6 L:D photoperiod. On each test day one beetle was selected from each of the replicates (four beetles/treatment; 2 female, 2 male). After the flight test was completed the beetles were returned to the lab for dissection and muscle preservation for ultrastructure analysis by transmission electron microscopy. Testing was conducted on day 5, 8, 11, and 15 for Test 1; day 5, 8, and 15 for Test 2 and day 5, 8 and 15 for Test 3.

Sample preparation. The eight beetles that had been used in each flight experiment were prepared simultaneously for ultrastructural analysis. All samples were prepared at room temperature. The following protocol was used for all beetles: The elytra, head and abdomen were removed from the beetles and the entire thorax was placed in an epindorf tube of 2% glutaraldehyde in 0.1M sodium phosphate buffer (SPB) solution. After 1 h the thorax was removed and placed in a 0.1M solution of SPB for dissection of the dorso-longitudinal indirect flight muscles. The muscles, attached to one apodeme, were put into a fresh solution of 2% glutaraldehyde, in 0.1M SPB, for one more hour. All vials containing tissue were rotated to maintain constant, gentle agitation. The muscles were washed three times in 0.1M SPB and transferred to a 1% osmium tetroxide/0.1M SPB solution for 1 h. This was followed by another set of 0.1M SPB washes followed by three distilled water washes. The tissue was en bloc stained for one hour in a saturated solution of uranyl acetate, before dehydration in a graded ethyl alcohol series. An acetone transition was used to facilitate infiltration with a mixture of Epon-Araldite-Spurrs epoxy resin (Klomparens et al. 1986). Ultrathin sections of a muscle were obtained for observation using a Philips 201 transmission electron microscope. Ultrathin sections, on a copper grid, were treated with a 1% solution of disodium EDTA before post-staining with uranyl acetate (30 min) and lead citrate (5 min). Micrographs were obtained of muscle tissue from both fed and starved beetles on each test day. Descriptions of the micrographs were made using a rating scale of 0-5 for overall status of the ultrastructure: 0=lacking visible fibrils, with very little sarcoplasm; 1=trace of fibrils, little sarcoplasm, large t-tubule spaces; 2=convoluted basal lamina, minimal mitochondria, fibrils small, empty spaces; 3=started disintegration of fibrils, loss of 'integrity' of the cell; 4=intact fibrils, fibrils farther apart, many mitochondria, some space between mitochondria and fibrils; 5=no cellular disorganization, tightly compacted fibrils, many mitochondria. Four to five micrographs, of fibers representing different areas of the tissue for each beetle, were examined in a blind analysis to determine qualitative degeneration. Measurements were also made of fibril diameter (5 fibrils sampled/replicate). Two replicates for fed beetles and three to four replicates for starved beetles were examined. Fibril diameter was measured for a quantitative analysis of muscle degeneration in fed compared to starved beetles. Statistical analysis was done on fibril diameter with a t-test, $p=0.05$.

Results

The dorso-longitudinal indirect flight muscle in the starved post-diapause Colorado potato beetle degenerated between 5 and 8 days after the onset of starvation. These muscles exhibited structural changes similar to those that have been characterized for degenerating insect muscles under a variety of physiological conditions. A similar process of degeneration and ultrastructural changes occurred in each of the three tests conducted. The difference between these three tests was the storage time of the adults from collection in the field to initiation of treatment.

Quantitative measures of fibril diameter showed comparable values for starved and fed beetles at day 5, with the values for fed beetles remaining consistent throughout the period and those for starved beetles declined. Test 1 (Figure 1) showed no significant difference between fibril diameters for fed (2.28 µm) and for starved (2.15 µm) beetles on the first sample date (day 5) (t-test, $p>0.05$). Mean fibril diameters of fed (2.56 µm) and starved (1.75 µm) beetles were significantly different (t-test, $p<0.05$) at day 5 for test 2 (Figure 2) primarily due to a single beetle which showed more advanced stages of degeneration inconsistent with the other beetles. This individual may have been less fit than other beetles before the start of the test. Test 3 (Figure 3) showed no significant difference between fibril diameters for fed (1.91 µm) and starved (2.16 µm) beetles at day 5 (t-test, $p>0.05$).

From day 5 muscle degeneration was seen in the starved beetles. The mean fibril diameter for fed beetles at day 8 was 2.01, 2.17 and 2.44 µm (test 1, 2, 3, respectively) and 0.94, 1.24 and 1.23 µm, respectively, for the starved beetles (significantly different at $p<0.001$, t-test). Significant differences between the fibril diameters of fed and starved beetles were maintained for all three tests on day 10/11 and day 15 (see Appendix for mean fibril diameter values).

At day 11 for test 1 and day 10 for test 2 (test 3 was not sampled at day10/11) the ultrastructural changes that occurred were similar to those of the starved treatment at day 8. The mean fibril diameter of starved beetles was 1.33 and 1.31 µm (test 1 and 2 respectively) compared to 2.9 and 2.13 µm for fed beetles (test 1 and 2) (Figure 1, 2) (significantly different at $p<0.05$, t-test).

Samples from starved beetles taken at day 15 showed similar degenerative fibrils, as previously described. The mean fibril diameter of starved beetles was 1.06, 1.54 and 2.17 µm compared to 2.27, 2.58 and 2.87 µm for the fed beetles at day 15 (test 1, 2, and 3 respectively) (Figure 1, 2, and 3) (significantly different at $p<0.001$, t-test).

Qualitative measures, based on a descriptive scale of overall fiber status (see Methods and Materials), indicated that there was a progressive degeneration and disorganization of cellular contents in starved beetles. Test 1 and 2 (Table 1 and 2) showed a progressive disorganization of the cellular contents as well as changes in structural appearance of the cell and its contents, from day 5 through day 15. In test 3 (Table 3) there was a similar change from day 5 to day 8 but at day 15 the fiber structure resembled that of the fed beetles.

Table 1. Test 1 Qualitative rank of fiber organization for dorso-longitudinal indirect flight muscles in post-diapause starved and fed Colorado potato beetles.

Treatment		**Day**		
	5	**8**	**11**	**15**
Fed	5	4	5	4
	5	5	5	4
Starved	5	2	2-3	3
	5	5	3-4	2
	5	2.5	3	3
	5	2	2	1

Rank 0-5 of overall muscle fiber integrity and structure: 0=lacking visible fibrils, very little sarcoplasm; 1=trace of fibrils, large spaces, little sarcoplasm; 2=convoluted basal lamina, minimal mitochondria, fibrils small; 3=losing "integrity "of the cell and "disintegration" of fibrils; 4=intact fibrils, fibrils farther apart, many mitochondria; 5=no disorganization, tightly compacted fibrils, many mitochondria.

Table 2. Test 2 Qualitative rank of fiber organization for dorso-longitudinal indirect flight muscles in post-diapause starved and fed Colorado potato beetles.

Treatment	**Day**			
	5	**8**	**10**	**15**
Fed	5	5	5	4
	5	5	5	4
Starved	4-4.5	3	4	2-3
	4	4	1	1
	2	2	4	4.5
	5	5	1-2	

Rank 0-5 of overall muscle fiber integrity and structure: 0=lacking visible fibrils, very little sarcoplasm; 1=trace of fibrils, large spaces, little sarcoplasm; 2=convoluted basal lamina, minimal mitochondria, fibrils small; 3=losing "integrity" of the cell and "disintegration" of fibrils; 4=intact fibrils, fibrils farther apart, many mitochondria; 5=no disorganization, tightly compacted fibrils, many mitochondria.

Table 3. Test 3 Qualitative rank of fiber organization for dorso-longitudinal indirect flight muscles in post-diapause starved and fed Colorado potato beetles.

Treatment	**Day**		
	5	**8**	**15**
Fed	4	4	4
	4	5	4
Starved	5	2	5
	4-5	3	4
	4	4	3-3.5
	3		4

Rank 0-5 of overall muscle fiber integrity and structure: 0=lacking visible fibrils, very little sarcoplasm; 1=trace of fibrils, large spaces, little sarcoplasm; 2=convoluted basal lamina, minimal mitochondria, fibrils small; 3=losing "integrity" of the cell and "disintegration" of fibrils; 4=intact fibrils, fibrils farther apart, many mitochondria; 5=no disorganization, tightly compacted fibrils, many mitochondria.

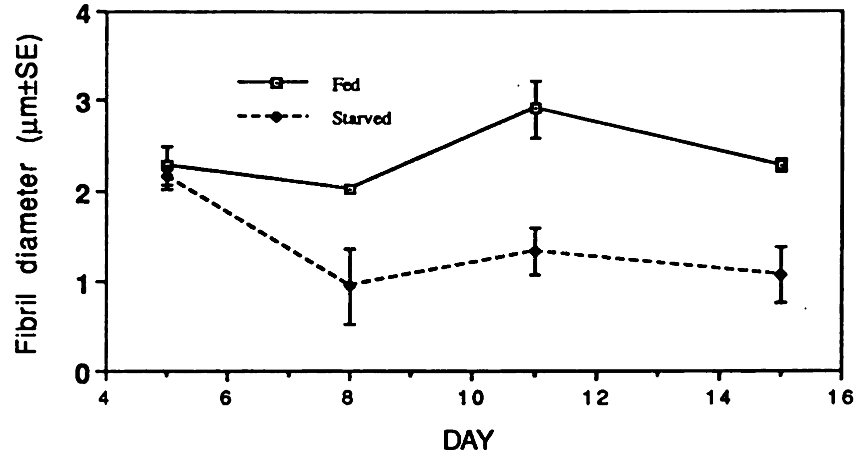

Figure 1. Test 1. Mean fibril diameter (µm±SE) in the dorso-longitudinal muscle of fed and starved post-diapause Colorado potato beetles.

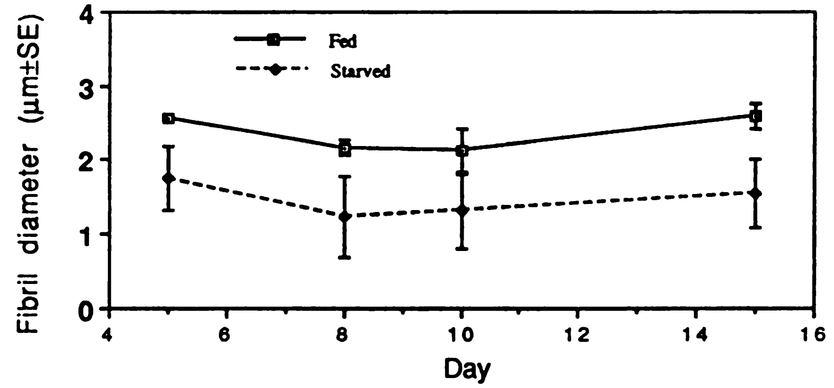

Figure 2. Test 2. Mean fibril diameter (µm±SE) in the dorso-longitudinal muscle of fed and starved post-diapause Colorado potato beetles.

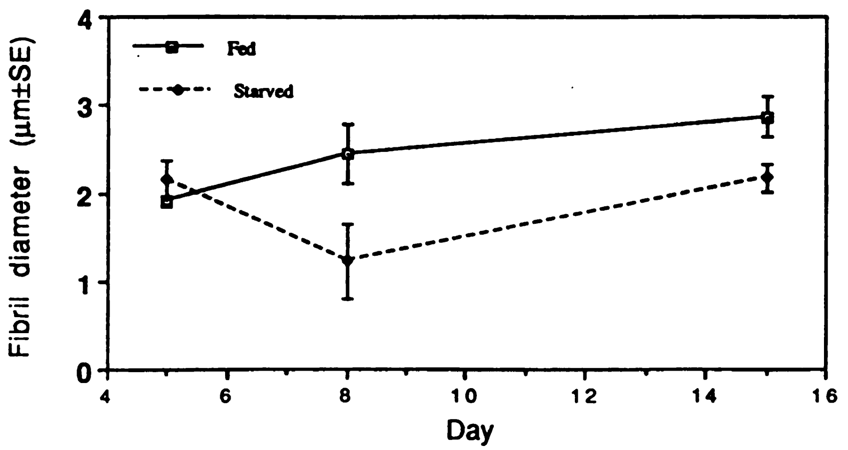

Figure 3. Test 3. Mean fibril diameter (µm±SE) in the dorso-longitudinal muscle of fed and starved post-diapause Colorado potato beetles.

The fiber ultrastructure of fed and starved beetles was comparable after 5 days of treatment. The fiber resembled a typical fibrillar insect muscle with concentric, organized fibrils, containing a regular pattern of actin and myosin filaments (Figure 4 and 5). There was little, if any, empty space in the sarcoplasm. The sarcosomes were typically large and generally irregularly shaped. The cristae of the sarcosomes were regular and clearly distinguishable. The sarcosomes occupied much of the space between the fibrils and the tracheoles were in close apposition to the sarcosomes and the fibrils, indicating efficient transfer of oxygen to the cell.

The fiber ultrastructure of the fed beetles was similar from day 5 to day 15 (Figure 6,7, and 8). By day 8 of starvation; however, degeneration started to occur. Changes indicative of degeneration were seen in various cell structures. The mitochondria of starved beetles were affected and at day 8 they were often smaller, more compact and circular or oval, with the cristae less visible or not at all apparent (Figure 9). There were other electron dense bodies in the sarcoplasm of the starved beetles. They were often irregularly shaped and either uniformly electron dense or had patches of greater electron density than the rest of the body (Figure 9). Tracheoles were evident in the sarcoplasm; however many of them were collapsed.

With a decrease in fiber area the basal lamina began to fold, giving a convoluted appearance, and in some areas the sarcolemma had separated from the basal lamina. (Figure 9 and 10).

A disintegration of the fibrils was apparent, as well as a decrease in fibril diameter. The fibril lost the protein contractile elements

Figures 4-15

Transmission electron micrographs of ultrathin cross sections of dorso-longitudinal indirect flight muscles from starved or fed Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Samples were fixed in glutaraldehyde and osmium tetroxide, en bloc stained with uranyl acetate and post-stained with uranyl acetate and lead citrate.

Abbreviations: f=fibrils
m=mitochondria
Tr=tracheoles
t=t-tubules
e=electron dense body
P=phagocytic cell
BL= basal lamina
s=sarcolemma
L=lipid

Figure 4: Cross section from a fed beetles after 5 d of treatment. The fibrils (f) are big with large irregular mitochondria (M) tightly packed between the fibrils. Bar=0.5 µm.

Figure 5: Cross section from a starved beetle after 5 d of treatment. The fibrils (f) are large and tightly packed by mitochondria (M). Bar=0.5 µm).

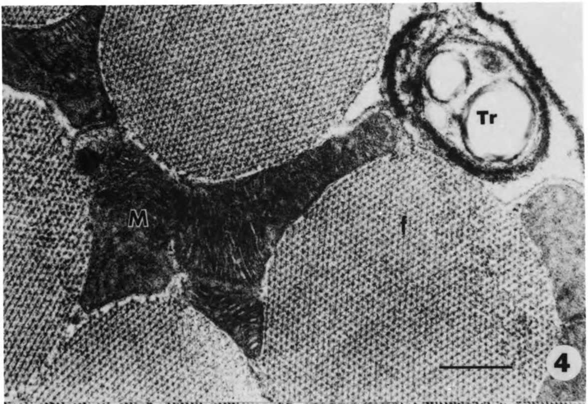

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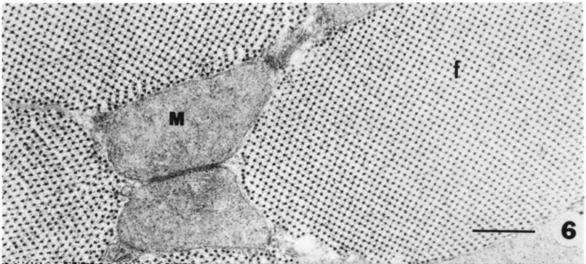
Figure 6: Cross section from a fed beetle after 8 d of treatment. f=fibril, M=mitochondria. Bar=0.5 µm.

Figure 7: Cross section from a fed beetle 10 d after treatment. f=fibril, M=mitochondria. Bar=0.5 µm.

Figure 8: Cross-section from a fed beetle 15 d after treatment. f=fibril, M=mitochondria. Bar=0.5 µm.



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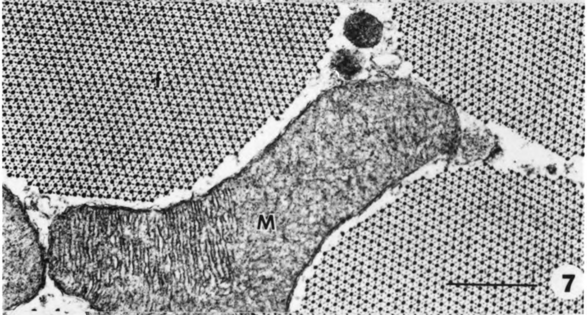

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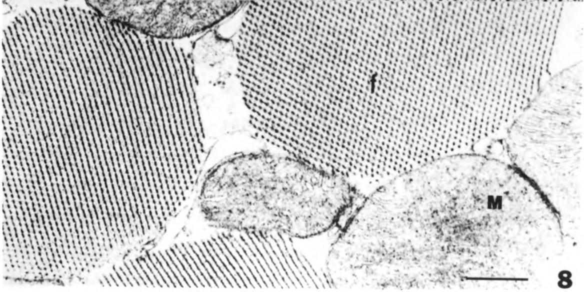

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while taking on a disorganized appearance. Both actin and myosin myofilaments "disappeared" from either the edge or the center of the fibrils. This left the fibrils with irregular "holes" in them, devoid of any myofilaments, giving the fibrils a general scattered appearance (Figure 11).

The appearance of opaque bodies started to occur in the sarcoplasm after 8 days of starvation. These bodies appeared randomly in the sarcoplasm and varied in size. They were usually round and were not membrane bound. Lipid droplets were only observed inside the muscle cell; although in one instance it appeared as though a lipid droplet was being exocytosed through the sarcolemma (Figure 10 and 11). The exocytosing lipid droplet also contained some electron dense areas and a clear area.

Clumps of heterochromatin were observed along the edges of the nuclei in the starved beetles (Figure 12) at day 8.

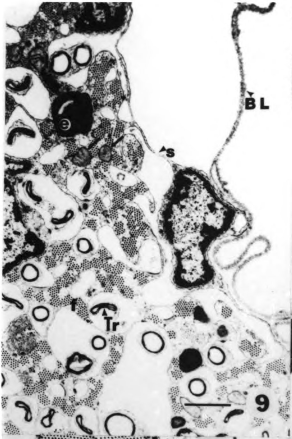
At day 11, the electron dense bodies were abundant in the sarcoplasm (Figure 13) While fibrils in some replicates still had an organized appearance, others were very disorganized. The myofilaments of some fibrils appeared "scattered" as the integrity of the fibril structure was lost; this included a dissolution of myofilaments (Figure 13). In some areas the actin filaments have disappeared, leaving the myosin filaments. In other areas myosin filaments diappeared leaving actin filaments. At this stage of degeneration the opaque lipid bodies were still evident and appeared to be more abundant. At day 10/11 the shrinkage of the cell and reduction of the sarcoplasm had increased the large extracellular spaces of the t-tubules (Figure 14). There were still mitochondria

Figure 9: Cross section of a muscle fiber from a beetle starved 8 d. There are several electron dense bodies (e) in the sarcoplasm.The mitochondria are small and compact (indicated by arrows). Some tracheoles have collapsed (Tr) and the sarcolemma(s) has separated from the basal lamina(BL). f=fibrils. Bar= 1.0 µm.

Figure 10: Cross section of a muscle fiber from a beetle starved 8 d. A lipid droplet (L) is seen to be apparently exocytosing from the sarcoplasm. The basal lamina(BL) shows the convolutions and is separated from the sarcolemma(s). f=fibril. Bar=1.0 µm.

Figure 11: Cross section of a muscle fiber from a beetle starved 8 d. Several lipid droplets(L) are seen in the sarcoplasm. f=fibrils and the arrows indicate the dissolved myofilaments from the periphery and from the center of the fibrils. M=mitochondria. Bar=1.0 µm.

Figure 12: Cross section of a muscle fiber from a beetle starved 8 d. The nuclei (N) show condensed patches of heterochromatin. M=mitochondria which are very small. Bar=1.0 µm.



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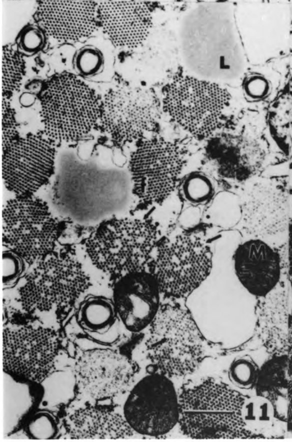

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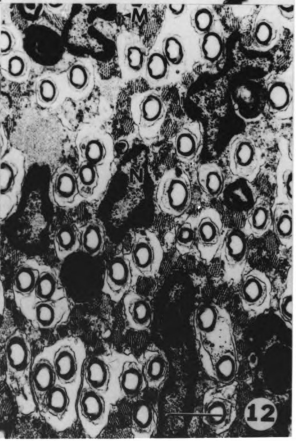

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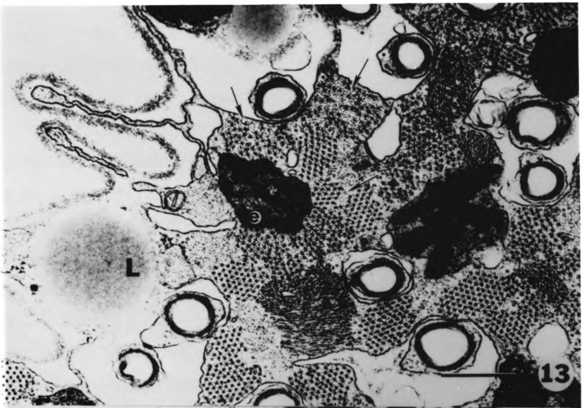
Figure 13: Cross section of a muscle fiber from a beetle starved 10 d. Several electron dense bodies (e) are evident in the center of the micrograph. The remnants of the fibrils show the actin and myosin filaments scattered in the sarcoplasm (arrows) There are several lipid bodies (L) present in the sarcoplasm. Bar=0.5 μm.

Figure 14: Cross section of a muscle fiber from a beetle starved 10 d. Along the edge of the basal lamina is a phagocytic cell (P). There is a large electron dense body inside the phagocytic cell that resembles the dense bodies in the sarcoplasm. The t-tubule spaces (t) are very dilated. M=mitochondria. f=fibril. BL=basal lamina. Bar=1.0 μm.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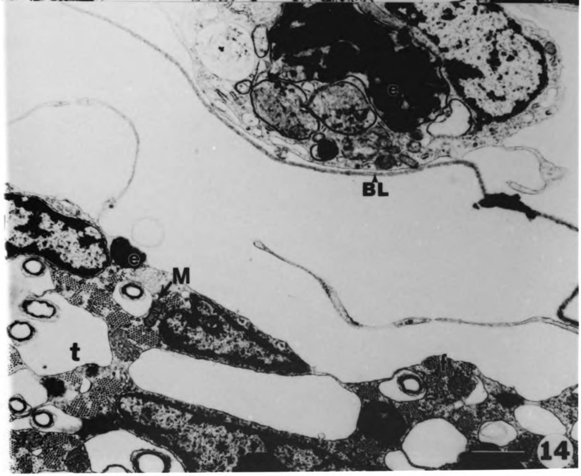
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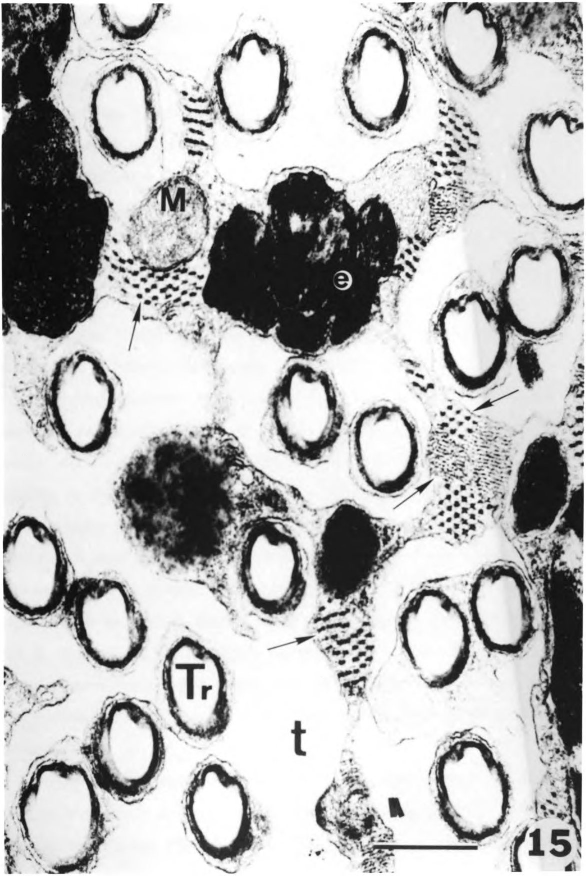
scattered in the sarcoplasm but they appeared to be fewer in number and were very small. There was evidence, at day 10/11, of what appeared to be a phagocytic cell (Figure 14).

Day 15 in the starved beetles showed advanced stages of cellular disorganization. There were remnants of fibrils with some actin and myosin filaments scattered in the sarcoplasm. Mitochondria had not completely disappeared but were few in number and very compact in shape (Figure 15). In very degenerated samples there was very little sarcoplasm remaining, with large spaces formed by the t-tubules occupying much of the area of the cross-section (Figure 15) Large bodies of varying electron density were also apparent after 15 days of starvation. These are difficult changes to quantify; therefore, an estimation of the relative change from day 10/11 was difficult to make.

Figure 15: Cross section of a muscle fiber from a beetle starved 15 d. There are several electron dense bodies(e) of varying degrees of density. Expanded t-tubules(t) occupy most of the area of the cross section. The myofilaments are few and are scattered in the remnants of the sarcoplasm (arrows). Tr=tracheole. M=mitochondria. Bar=0.5 µm.



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Discussion

Fibril diameters of starved and fed beetles, at day 5 (1.75 to 2.56 µm), were similar to those obtained by Kort (1969) for fully developed dorso-longitudinal muscle fibrils of Colorado potato beetle under long-day conditions. He reported fibril diameters of 2 µm 4 days after adult emergence from pupation, and a maximum of 2.4 µm after 8 days of development. My values of 2.28, 2.56 and 1.91 µm (test 1, 2, 3 respectively) for the fed beetles and 2.15, 1.75 and 2.16 µm, respectively, for starved beetles at day 5 were similar to those of a fully mature muscle in summer adults.

The fibril diameter in starved beetles was significantly reduced, compared to the fed beetles, at day 8. Stegwee et al. (1963) showed a similar decrease in fibril diameter in the dorso-ventral indirect flight muscles in diapausing Colorado potato beetle. Although no measurements were given, they noted a reduction in the width of the fibrils 8 d after the beetles entered diapause. I did not sample between 5 and 8 d after starvation; however, I observed a similar degeneration in starved beetles, to that obtained by Stegwee et al, at day 8. Atkins and Farris (1962) observed, on a gross structural level under a stereoscopic microscope, the reduction in width of the dorso-longitudinal indirect muscles of scolytid beetles after 10-15 d in the galleries.

Flight muscle degeneration in aphids was seen to begin within 2-3 days of the aphids settling on hosts to feed (Johnson 1957). Within 4 days of introducing adult Ips into the bark, muscle degeneration started to occur (Bhakthan, Borden and Nair 1970). It appears, from the literature, that adult flight muscle degeneration occurs at different times depending on the insect and/or other physiological conditions. In Colorado potato beetle, however, the rate of degeneration appears consistent whether the cause is diapause-induced degeneration (Stegwee et al 1963) or starvation. Atkins and Farris (1962) also noted gross muscle structure degeneration of both male and female scolytid beetles after 21 days of starvation. This is, to my knowledge, the only other documented examination of the effects of starvation on muscle degeneration; because these observations were done on a more gross morphological scale I cannot compare them to the ultrastructural details of degeneration in my study.

Some of the same phenomena that occurred in my experimentally-induced degeneration were similar to the normal, generalized degenerative process that occurs in metamorphosing insects and in degeneration of adult insect muscle (Rees and Usherwood 1972, Jahromi and Bloom 1979, Johnson 1980 and Lockshin and Beaulaton 1974, Beaulaton and Lockshin 1977, Unnithan and Nair 1977, Rheuben, in press). The fiber cell undergoes a process of overall structure degeneration and disorganization. This was seen in the cell membranes, the sarcolemma and the basal lamina, and in the cellular contents, including the mitochondria, the nuclei and in the contractile proteins, actin and myosin. The presence of bodies such as the electron dense bodies and lipid bodies, which are not present in normal muscle cells, were also noted in the degenerated muscle of starved beetles.

The basal lamina of starved beetles became convoluted in appearance with the shrinkage of the the sarcoplasm. The sarcolemma was also extended into folds and in some areas was separated from the basal lamina. Auber-Thomay (1967), in Rhodnius, and Randall and Pipa (1969), in Galleria mellonella, noted infoldings of the basal lamina and local dissociation of the basal lamina and the sarcolemma. There has been no explanation in the literature for the cause of the separation of the sarcolemma from the basal lamina. This may be induced merely by mechanical causes due to the shrinkage of the sarcoplasm or to other chemical processes. There was also the appearance, in starved Colorado potato beetles, of what has been referred to in the literature as swollen or dilated t-tubules (Beaulaton and Lockshin 1977, Randall and Pipa 1969, Rheuben, in press). The sarcoplasm appears to shrink away from the tracheolar evaginations giving a swollen appearance to the t-tubules. There was also evidence of collapsed tracheoles; although not all tracheoles appeared collapsed. Collapsed tracheoles would seem to indicate that there to be a breakdown of the taenidia. Rheuben (in press) and Randall and Pipa (1969) showed collapsed tracheoles in muscle degeneration of metamorphosing Lepidopteran species.

The actin and myosin filaments were also affected by induced starvation of the Colorado potato beetles. These contractile proteins disappeared, causing a loss of the fibril integrity and a scattered appearance of actin and myosin. Johnson (1980) described a dissolution of myosin filaments, in aphids, both at the periphery of the fibril and in channels progressing towards the interior of the fibril. He suggested, from the pattern observed in aphids, that dissolving filaments in some way influenced adjacent filaments causing them to break down. Beaulaton and Lockshin (1977) observed the dissolution of the thick filaments in an apparently random fashion within fibrils of two Lepidopteran species. Even though erosion of myofilaments, in Lepidoptera, occurred for both thick and thin filaments, the thick filaments appeared to be the first to be digested. These authors, in their investigation of degenerating intersegmental muscles of the newly emerged adult moth, suggested that the dissolution of the myofilaments serves the adaptive function of providing amino acids for the non-feeding adult insect. The pattern of dissolution of degenerating flight muscles in starved Colorado potato beetles was similar to that described for those Lepidopteran species and for the aphids. Experimentally-induced starvation, in the Colorado potato beetle presents a similar situation to the naturally non-feeding adult Lepidoptera. The starved beetles could also have been using the digested protein as a source of energy. While I observed a similar dissolution of the filaments, I do not have any evidence for the mechanism by which this happens.

Large clumps of heterochromatin were observed along the edges of the nuclei in starved beetles. This was particularily apparent at day 8 of starvation but was not evident to the same extent in the later stages of degeneration. Auber-Thomay (1967) observed a decrease in muscle nucleur size in Rhodnius, with the chromatin appearing in clumps in the nuclei. Beaulaton and Lockshin (1977) also noted, in later stages of metamorphic muscle degeneration in Lepidoptera, that the nuclei contained condensed patches of chromatin. I did not notice any apparent decreases in nuclear size as was observed in the Rhodnius study. There is no evidence for why the chromatin would appear in large clumps during degeneration.

Opaque bodies, similar to what has been described in the literature as lipid bodies were apparent in the sarcoplasm of the starved Colorado potato beetle after day 8 and 10/11. These bodies have been described in muscle degeneration in adult Ips, caused by application of a juvenile hormone analog (Unnithan and Nair 1977). They have also been observed in hormonally-regulated muscle degeneration, but they are not typically seen throughout the degenerative process. Rheuben (in press) observed similar lipid droplets in the muscle of metamorphosing Manduca. Lockshin and Beaulaton (1974) label these droplets 'liposomes'. Crossley (1968) identified similar bodies, by histochemical analysis, to be lipid droplets in the pupae of Calliphora. He observed them in the cytoplasm of phagocytic hemocytes and floating in the hemolymph adjacent to degenerating muscle. This might seem to indicate the exocytosis of the lipid droplets from the sarcoplasm (Crossley 1968). In my study there was an example of apparent exocytosis of a lipid droplet from the sarcoplasm in the starved beetles at day 8; there was also electron dense material in the exocytosing lipid droplet. It is not clear what these are. In most samples the lipid bodies were only seen inside the muscle cell.

Beaulaton and Lockshin (1977) noted the apparent exocytosis of lipid droplets in the degenerating intersegmental abdominal muscles of the newly emerged adult moth. They suggested that this illustrated a mobilization of substances which, once released into the hemolymph, could be a source of energy for the non-feeding adult insect. The utilization of the lipid bodies as a source of energy would have been beneficial to the starving Colorado potato beetle.

Parts of the liposomes in the beetle fibers were sometimes clear. Rheuben (in press) found that liposomes in the degenerating muscles of metamorphosing Manduca sometimes showed clear cores. She suggested that this may reflect the solubility of the contents in the organic solvents used in sample preparation.

The mitochondria of the flight muscle were affected by starvation. In a fully developed fed beetle the mitochondria were very large, indicative of the high energy demand of flight muscles, with well developed and visible cristae. In starved beetles; however, the mitochondria were fewer in number, much smaller, more compact and round and the cristae were not always obvious. de Kort (1969) found that newly emerged summer adults had small mitochondria with few cristae. He also determined that the enzyme activity of the inner membrane was also low. Lin, Hudson and Strickland (1969) observed in dystrophic vertebrate muscle that there was an impaired capacity of mitochondria to oxidize pyruvic acid and fatty acid. They observed a marked reduction of mitochondria in the muscle fiber and postulated that the impairment of fatty acid oxidation may be due to a fundamental structural defect in the mitochondria. Despite the reduction in numbers and change in appearance of mitochondria in starved Colorado potato beetle, the extent of mitochondrial dysfunction is not known.

Johnson (1980) found a similar degeneration in the sarcosomes of aphid muscle. As with the starved Colorado potato beetle, the numbers of mitochondria decreased as degeneration proceeded. He suggests that the reduction in the numbers of mitochondria appears to be due to them being digested in vacuoles. It is not clear from my studies whether any vacuolar digestion occurred in the mitochondria of starved beetles.

The variation in stages of degeneration seen amongst the starved beetles indicates that the process of degeneration advances at a varying rate amongst individual beetles. Johnson (1959) noted the uneven progression of muscle breakdown in the adult flight muscles of an aphid after settling. He observed in the early as well as late stages of muscle breakdown that individual sarcomeres within and between fibrils varied in the degree of degeneration. Jahromi and Bloom (1979) noted differential degeneration in tenotomized locust retractor muscle. Lockshin and Beaulaton (1974) also observed, in metamorphosing muscle degeneration, that at all times during histolysis there was sufficient variability amongst fibers to suggest an asynchrony in the breakdown process.

The differential rate of muscle breakdown within an individual and amongst some individuals illustrates that this process occurs at a variable rate for a number of different physiological conditions. In some Scolytids there is a differential in the rate of muscle breakdown in relation to the sex of the beetle and linked to the reproductive status of the females (Atkins and Farris 1962). In Colorado potato beetle there does not appear to be a pattern of degeneration dependent on beetle sex. I did not monitor the status of ovary development in females; therefore, I cannot ascertain any potential correlation between reproductive status and flight muscle degeneration. It would be interesting to determine any potential correlations between the reproductive status of the female Colorado potato beetle and the flight muscle ultrastructure.

The overall trend of degeneration in starved Colorado potato beetles was, however, consistent within and between tests. The fibril diameter, as one indicator of muscle degeneration, started to decrease between day 5 and 8 and despite some variation amongst individuals there was a significant difference between the fed and the starved beetles. The qualitative measure of overall fiber integrity and structure also indicated degeneration in the starved beetles. The degenerative ultrastructural changes occurring in my experimental animals were similar to those changes described in the literature for normal muscle breakdown in metamorphosing insects as well as for other experimentally-induced and non-experimentally-induced muscle degeneration in adult insects.

The status of flight muscles in Colorado potato beetle has implications for their ability to disperse and to colonize new fields of host plants. As beetles emerge from diapause in the spring they are potentially faced with a period of starvation if the potato plants, or other suitable hosts, do not emerge at the same time. My data indicate that after 5-8 days of starvation the flight muscles have started to degenerate. The dispersal capability would likely decrease as the contractile elements continued to degenerate. From a management perspective this could be useful in potato-growing regions where rotation was used. Potato fields that are rotated with another crop on alternate years can contain large populations of over-wintering beetles when the non-potato crop is planted. When the post-diapause beetles emerge in the spring into a field that has been planted with an alternate crop, starvation will result in degenerate muscle with a reduced flight capability. The beetles' inability to disperse long distances by flight could keep them in the rotated field or limit dispersal to short flights or walking, thus reducing the beetle population pressure on potato fields. Various mechanical, chemical and/or cultural control measures could be used to suppress the population in rotated fields.

Conclusions

Despite the fact that Colorado potato beetles are not known to be strong fliers, they are known to disperse by flight in the spring when they have emerged from diapause (Caprio and Grafius 1990, Voss and Ferro 1990b) and occasionally in the fall. The two studies presented here, although separate, were linked in design and in execution. The intention of this connected method of approach was to try to establish a correlation between the ability of the starved and fed post-diapause Colorado potato beetle to fly and the ultrastructure of the dorso-longitudinal indirect flight muscles. These muscles are responsible, in beetles and in several other orders of insects, for much of the power of flight. They govern the up and down motion of the wings during flight. Two groups of muscles work antagonistically, with the contraction of the dorso-longitudinal muscles causing the downstroke of the wing and the alternate contraction of the tergo-sternal muscle causing the upstroke of the wing. These muscles, given their importance in flight, seemed a suitable group of muscles for ultrastructural study.

Some inferences can, therefore, be made concerning the relationship between the structure of this muscle and its function as determined by the responses in flight tendancy of starved and fed beetles. A more direct correlation between structure and functional capabilities could be made through electrophysiological studies on isolated muscle and through a quantitative method of determining flight sustainability of an intact individual. Even though the abovementioned types of studies were not done here, I can still draw some relationship between the results of these two studies that may prove helpful.

The ultrastructure of the dorso-longitudinal indirect flight muscles in starved Colorado potato beetle started to degenerate between day 5 and day 8 of starvation. This included a reduction in the filbril diameter as well as a general disorganization and degeneration of cellular contents. The results of the field flight behavior experiment indicated that over the time period tested, which varied from one test to another, the response in the tendency of the starved beetles to fly did not differ from that of the fed beetles. The low numbers of beetles make it difficult to draw concrete conclusions; however, by looking at the pattern of flight for the starved beetles over the time period sampled (1-35 d) I can draw some parallels to the muscle analysis. In test 1, there was a sharp decline in fibril diameter of starved beetles between day 5 and 8 of starvation. This corresponds to the pattern of flight behavior in test 1. In the flight test, no flight was observed by starved beetles after day 5. In test 2, the muscle fibril diameter declined between day 5 and 8. In test 2, flight of starved beetles was seen on day 8, 10, and 15. In test 3 there was a similar pattern of fibril diameter degeneration between day 5 and 8; however there was an increase in fibril diameter at day 15 for the starved beetles. Starved individuals, in test 3, showed flight tendency on day 8 (2/4 beetles) and on day 15 (3/4 beetles). Some starved beetles also flew as late as day 26. Since the muscle diameter of starved beetles, on day 15, was close to that of the fed beetles this could explain the flight ability of the starved beetles at day 15.

Test 1 is consistent with what I would expect: when muscle mass decreases the flight ability of the beetles theoretically should decrease. Test 2 does not necessarily follow the expected response based on fibril diameter; I saw some flight when muscle mass had declined. Test 3, in part, does not follow the expected response since I saw flight on day 8 when there was a sharp decrease in fibril diameter. I have been unable to explain the increase in muscle fibril diameter at day 15 in test 3 but the flight response does correlate with this increase. The analyses of the muscle indicate there to be a potential reduction in performance capability of these muscles due to the nature of the degeneration: the loss of cellular integrity along with the loss of the functional units of contraction- the myofibrillar structure. It theoretically follows that if the insect is deprived of nutrients from which to synthesize the constituent proteins that form the contractile units of a muscle, the performance of that muscle would dramatically decline. The flight studies show, however, that even when the muscle ultrastructure has apparently declined some beetles still flew.

There could be several explanations for these observations: 1) the method of testing and individual variability. There was variability amongst the individuals in the degree of muscle degeneration and in flight tendency. The method of testing may have contributed somewhat to the discrepancy. The beetles were first subjected to the field flight behavior study and those beetles that were recaptured were returned to the lab for muscle analysis; those beetles that were not recaptured were replaced in the muscle analysis by individuals from the same treatment. There was not always, therefore, a direct comparison of flight tendency and muscle ultrastructure on the same individual. It may be that in the early stages of muscle degeneration, when there is some variability amongst individuals those starved individuals that did not have greatly degenerated muscles were the ones that flew, while those that had greatly degenerated muscles did not fly. Larger sample sizes would help to measure some of the variability. By somehow recapturing those individuals that flew farther, a direct comparison of flight muscle ultrastructure and flight tendency could be made on each individual.

2) There is also, no doubt, a behavioral component to flight. A behavioral response may incite a starved individual to initiate flight to displace itself from adverse conditions even when the muscle mass has started to decrease. Since I measured only the tendency (whether the treated beetles flew or not), and not the sustainability of that flight, I observed this behavioral component in the flight response. I do not know at what stage of muscle degeneration the beetle is no longer capable of any flight or how a decline in flight sustainability might proceed in conjunction with a decline in muscle mass. A more conclusive study quantifying the length and duration of the flight ability of this insect in direct correlation with the individual's muscle ultrastructure would evaluate more fully the correlation between flight ability and muscle ultrastructure. An associated biochemical analysis of energy production and utilization in the flight muscles would also help to create a better understanding of this obviously complex relationship. As Skinner et al. (1983) pointed out, there are several areas in which research is critically needed to understand the role of movement in elucidating the

dynamics of pest species. One of these suggested areas is the identification of physiological and behavioral mechanisms conducive to initiation and maintenance of flight.

This study has established the degeneration pattern of the dorso-longitudinal indirect flight muscle ultrastructure of starved post-diapause Colorado potato beetle. The companion flight behavior study has enabled me to draw some correlation between the ultrastructure and flight tendency in starved and fed beetles. By fully understanding the structure/function relationship of Colorado potato beetle muscle and flight capability we would be better able to design effective management strategies to manipulate the dispersal of this complex and destructive pest. Because Colorado potato beetle has become resistent to virtually all insecticides, it is critical that cultural and mechanical controls be optimized to both suppress pest populations and prevent the spread of resistent genes. Further studies, expanding on this one, to elucidate the performance *capabilities* of the dispersal prone generations could contribute significantly to an integrated management plan for Colorado potato beetle.

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APPENDIX

Appendix

Mean fibril diameters of dorso-longitudinal muscles in CPB.

Table 4. Test.1 Mean fibril diameter (µm ±SEM) of dorso-longitudinal indirect flight muscles of fed and starved Colorado potato beetle.

Treatment	Day			
	5	8	11	15
Fed	2.28 ±.21	2.01 ±.01	2.90 ±.33	2.27 ±.0
Starved	2.15 ±.13	.94 ±.41	1.33 ±.26	1.06 ±.31

Table 5. Test.2 Mean fibril diameter (µm ±SEM) of dorso-longitudinal indirect flight muscles of fed and starved Colorado potato beetle.

Treatment	Day			
	5	8	10	15
Fed	2.56 ±.04	2.17 ±.09	2.13 ±.28	2.58 ±.17
Starved	1.75 ±.43	1.24 ±.54	1.31 ±.51	1.54 ±.46

Table 6 Test.3 Mean fibril diameter (µm±SEM) of dorso-longitudinal indirect flight muscles of fed and starved Colorado potato beetle.

Treatment	Day		
	5	8	15
Fed	1.91 ±.06	2.44 ±.34	2.87 ±.23
Starved	2.16 ±.22	1.23 ±.42	2.17 ±.15