STUDIES ON CONTROL OF THE ALFALFA WEEVIL, *HYPERA POSTICA* (GYLL.), IN OKLAHOMA WITH HYMENOPTEROUS PARASITOIDS

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Bachelor of Arts

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Russellville, Arkansas

1972

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1974

STUDIES ON CONTROL OF THE ALFALFA WEEVIL,

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PREFACE

This investigation culminated a three year study of biological control of the alfalfa weevil, *Hypera postica* (Gyllenhal), with Hymenopterous parasitoids. Much of the study was concerned with the distribution and rate of parasitism of *B. curculionis* in major alfalfa growing areas of the state. An investigation was also made into the effects of encapsulation of *B. curculionis* eggs and larvae by weevil larvae of mixed strains in Oklahoma. A third objective was to introduce, release, and establish other Hymenopterous control agents in Oklahoma. Releases were made of *Tetrastichus incertus* (Ratzeburg), *Microctonus aethiops* Nees; *Peridesmia discus* (Walker); and *Bathyplectes anurus* (Thompson). Samples were taken weekly from the release fields and rates of parasitism were determined once establishment was ascertained.

I would like to express sincere gratitude to the author's major adviser, Dr. Richard C. Berberet, associate professor, Department of Entomology, for his advice and cooperation throughout this study and in preparation of this manuscript. I am grateful to Dr. Berberet for providing financial assistance for this study through the USDA and the Oklahoma Agricultural Experiment Station.

Appreciation is expressed to the author's graduate committee members, Dr. Raymond C. Eikenbary and Dr. Jerry Young, for their advice and critical review of this manuscript. I would also like to thank Dr. Don C. Peters, Chairman of the Department of Entomology, for his assistance in providing a critical review of the manuscript.

I gratefully acknowledge the friendship, support, and assistance provided by Ms. Madeline Koch and Ms. Joan Hatfield both in the laboratory and outside the realm of this study.

Thanks is also expressed to Richard Wall, Doug Sander, and Neva Lyons for their unselfish assistance in the successful completion of this study.

This manuscript is dedicated to my mother, Mrs. Neva B. Gibson, who deserves unlimited thanks for being an inspiration to my family, a good friend, and most of all, a lovely Mom:

> We need to have PEOPLE who mean something
> to us, people to whom we can turn knowing
> that being with them is Coming Home.
>
> B. Cooke
> --Chantal

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LIST OF SYMBOLS

NE	Northeastern Oklahoma
NW	Northwestern Oklahoma
C	Central Oklahoma
SW	Southwestern Oklahoma
1HE	One Healthy Egg
2HE	Two Healthy Eggs
3HE	Three Healthy Eggs
1EE	One Encapsulated Egg
2EE	Two Encapsulated Eggs
(2EE)	Two Eggs in the same Capsule
1HL	One Healthy Larva
2HL	Two Healthy Larvae
1EL	One Encapsulated Larva
1HE + 1EE	One Healthy Egg and One Encapsulated Egg
1HE + 1HL	One Healthy Egg and One Healthy Larva
1HL + 1EE	One Healthy Larva and One Encapsulated Egg
1HL + 2EE	One Healthy Larva and Two Encapsulated Eggs
2HL + 1EE	Two Healthy Larvae and One Encapsulated Egg
1HL + (2EE)	One Healthy Larva and Two Eggs in same Capsule
1HL + 1EL	One Healthy Larva and One Encapsulated Larva
1EL + 1EE	One Encapsulated Larva and One Encapsulated Egg
1HE + 1HL + 1EE	One Healthy Egg plus One Healthy Larva plus One Encapsulated Egg

OTHER	Miscellaneous combinations infrequently encountered
% Eggs Encap	Percentage of Encapsulation in cases of Simple Parasitism
% Super Eggs Encap	Percentage of Encapsulation in cases of Super-parasitism
% Par Encap	Percentage of Encapsulation in all cases of parasitism
% Actual Par	Percentage of Weevil larvae parasitized regardless of condition (encapsulation) of parasitoid
% Effect. Par	Percentage of Weevil larvae parasitized with at least one healthy parasitoid

CHAPTER I

INTRODUCTION

The alfalfa weevil, Hypera postica (Gyllenhal) is an introduced pest which was first detected in the United States near Salt Lake City, Utah, in 1904 (Titus 1909). It spread slowly throughout the western States reaching California in 1923 (Essig and Michelbacher 1933) and, more recently, the western portions of the Dakotas, Nebraska, Kansas (Hagen and Manglitz 1967), and Oklahoma (Curry 1968). The weevil was not reported in the eastern United States until 1952, when it was discovered in Maryland (App 1959). By 1968 it had spread into 29 eastern states and much of the midwest (Brunson and Coles 1968).

The alfalfa weevil is now a major economic pest of alfalfa in most of its range which includes all 48 contiguous states and portions of Canada. The weevil has caused reduction in alfalfa acreage in several states; for example, Tennessee acreage declined 47,000 acres from 1964 to 1966, Georgia acreage decreased 35,000 acres from 1958 to 1965, and Alabama acreage dropped 188,000 acres from 1959 to 1966 (Cothran 1969). It has been estimated that over 50 million dollars are spent each year in the United States and Canada on control of this insect pest (Horn 1971).

The eastern and western populations of the alfalfa weevil in the United States are regarded as two distinct strains of H. postica. Partial sterility occurs when the two strains are crossed: eastern males crossed with western females produces viable offspring, while western males crossed with eastern females produces mostly infertile eggs (White et al. 1972).

The eastern strain was first reported in Oklahoma in 1968, when it was collected in Delaware and Sequoyah counties. It is thought to have entered Oklahoma from Arkansas and Missouri (Berberet and Flora 1973). The western strain is believed to have entered the state from western Kansas and eastern Colorado and to have maintained a static distribution in the northwestern corner. In 1969 the weevil was detected in the northwestern corner of Oklahoma in Harper County as well as in additional eastern counties. Because of the westward migration of the eastern strain of the weevil in Oklahoma and the apparent static distribution of the western strain in the northwestern corner of the state, it is believed that the two strains intermingled in the vicinity of Woods and Woodward counties sometime in 1969 or 1970. As of 1973, mainly the eastern strain and eastern-western hybrids existed in Oklahoma because of the cross-mating between eastern and western weevils as the eastern strain spread westward.

In Oklahoma the alfalfa weevil overwinters as an adult and becomes active on the warmer days of early spring when maximum temperature reaches 50°-60°F. These overwintered adults begin feeding and ovipositing on the small green alfalfa stems. Weevil eggs are inserted inside the alfalfa stems. The larvae emerge from the stems and ascend the plant to begin feeding in the terminals. Here the larvae pass through four instars before they drop to the soil surface to spin a cocoon and pupate. The larval period lasts two-four weeks while the pupal period lasts about one week. After the pupation period, adults emerge and begin feeding on alfalfa foliage. By late May or early June, most larvae have developed into adults which enter summer diapause (aestivation) from June through October. During unusually cool periods in the fall, many weevil adults will break diapause and commence feeding and ovipositing on the fall growth of alfalfa.

Insecticide application is the primary means of controlling the weevil in Oklahoma, and nearly all alfalfa acreage needs to be treated at least once per season. Because insecticide applications are quite costly, research directed toward development of alternate means of control has commenced in many states including Oklahoma. Current varieties of tolerant alfalfa are effective only when weevil infestations are light. The high population levels of the alfalfa weevil in Oklahoma since 1971 have caused damage that exceeds the ability of tolerant varieties to prevent economic loss.

Establishment of biological control agents of the alfalfa weevil in many areas of the United States has proven to be an important addition to other means of control. A complex of introduced Hymenopterous parasitoids has provided biological control of alfalfa weevil populations along the eastern seaboard (Miller et al. 1972), and one species, Bathyplectes curculionis (Thompson) has provided partial control of the weevil in some areas of the midwest (Wilson and Armbrust 1970) and California (van den Bosch 1964).

In 1911-14 B. curculionis was introduced into Utah from southern Europe as a biological control agent of the alfalfa weevil (Chamberlin 1926). Subsequent releases were made in Colorado in 1918; Nevada, 1921; California, 1933; and Oregon, 1934 (Clausen 1956). This parasitoid had spread throughout the range of the alfalfa weevil in the western states, moving into Idaho, Wyoming, Nebraska, Kansas (Hagen and Manglitz 1967) and Oklahoma (Berberet and Flora 1973) without the aid of augmentative releases. B. curculionis is generally considered to be an effective control agent of the alfalfa weevil in most western states, particularly in California (van den Bosch 1964) and in the Great Basin area (Hamlin et al. 1949).

The eastern strain of the alfalfa weevil reached damaging population levels by 1957 in many of the eastern states (App 1959). Because of its successful establishment in the western states, B. curculionis was introduced into the eastern states in 1958 as part of a biological control program for the weevil (Puttler et al. 1961). Through releases made in several states, it was established in most eastern states by 1968 (Brunson and Coles 1968). In the midwest this parasitoid was released against the eastern strain of the weevil in Tennessee in 1962-63, Kentucky in 1964, and Indiana in 1966-67 (Dysart and Puttler 1965). B. curculionis is given credit for partial control of the alfalfa weevil in Illinois and Indiana and has an important role in integrated control programs in the midwest (Wilson and Armbrust 1970).

Despite many releases, there is no evidence of economic control of the alfalfa weevil by B. curculionis alone in the eastern states. An explanation for this is given by Puttler (1967) who has shown the effectiveness of B. curculionis as a parasitoid of the alfalfa weevil to be reduced in eastern United States due to encapsulation of its eggs by haemocytes of weevil larvae. Fully encapsulated eggs invariably die, due apparently to interference by the haemocyte capsule with respiration and nutrition of the parasitoid embryo (van den Bosch 1964). This phenomenon was first reported in H. postica by van den Bosch (1964) in California in the western strain of the weevil. The ability to encapsulate B. curculionis eggs is scarcely expressed in the western strain of the weevil (van den Bosch 1964) (Salt and van den Bosch 1967).

The failure of B. curculionis to reduce weevil populations to below economic levels prompted further research and subsequent attempts to introduce numerous other biological agents for weevil control. In the years 1961-68, one or more of 10 species of parasitoids were released in 20 eastern states (Brunson and Coles 1968). Of those released, only four species in addition to B. curculionis have become established in eastern states: Bathyplectes anurus (Thompson), a larval parasitoid established in many eastern states; Microctonus aethiops Nees, a bivoltine adult parasitoid established in New Jersey and Maryland; Microctonus colesi (Drea) a larval-adult parasitoid established in six states; and Tetrastichus incertus (Ratzeburg), a gregarious, multi-brooded larval parasitoid also established in many eastern states (Brunson and Coles 1968). Other species released but not yet established include: Peridesmia discus (Walker), an egg predator; Microctonus stelleri Loan, a larval parasitoid; and Bathyplectes stenostigma (Thompson) another larval parasitoid.

The complex of five established parasitoids is credited with successful biological control of the alfalfa weevil in northeastern and middle Atlantic states (Miller et al. 1972). Berberet initiated a series of releases of Hymenopterous parasitoids and predators in Oklahoma during 1972 as a cooperative effort with the Insect Identification and Parasite Introduction Research Branch, ARS, USDA at Moorestown, New Jersey (Berberet and Flora 1973). Life cycles of three Hymenopterous parasitoids and one predator, which were released in Oklahoma as part of this effort and included in this study, are summarized below.

B. anurus is a parasitoid that completes one generation per year. Adult B. anurus emerge from overwintering cocoons at the time of peak abundance of weevil larvae each spring. The adults oviposit in first and second instar weevil larvae. The parasitoid larvae destroy their hosts and spin cocoons within those spun by the hosts. The dark mahogany cocoons, which possess a narrow white or cream-colored stripe about the midsection are about 4mm in length and 2mm in diameter. The cocoons can be easily distinguished from those of other species because of the ability of the parasitoid larva within each cocoon to flip, causing the cocoon to jump. B. anurus larvae aestivate in cocoons during the summer months, pupate in the fall, and overwinter as adults.

T. incertus parasitizes third and fourth instar weevil larvae during the late spring and summer in northeastern states. The parasitized weevil larva, which spins a cocoon before dying, exhibits a characteristic dark-brown mummified appearance. The diapausing parasitoid larvae of the final generation each season overwinter in host mummies. They pupate, and adults emerge during the following spring and summer. As many as 23 parasitoids have emerged from a single host (Horn 1971), however, an average of five parasitoids emerge per host (Brunson and Coles 1968).

A parasitoid of weevil adults, M. aethiops, is a bivoltine species which emerges from overwintered weevil adults when they become active in early spring. The first generation is completed in overwintered weevils, which feed and lay eggs in alfalfa in the early spring. The parasitoid larvae develop within the adult weevils, feeding internally and usually destroying the reproductive organs of the weevils. The larvae emerge from weevils, killing them in the process, and pupate within small gray cocoons in debris on the soil surface. Adult parasitoids then emerge from cocoons and begin a second generation in newly emerged adult weevils. First instar parasitoid larvae of this second generation diapause within aestivating weevil adults until the following spring when they complete development and emerge. This parasitoid is unusually effective because before it kills its host adult upon emergence, it effectively casterates sexually mature weevils (Drea 1968). This characteristic allows the parasitoid to reduce the repoductive potential of a population of weevil adults before they are actually killed.

Little is known about the life cycle of P. discus except its method of preying upon the alfalfa weevil. This species deposits eggs within clusters of weevil eggs in alfalfa stems and the resulting larvae destroy the weevil eggs. It is active during the winter and early spring in southern Europe (Chamberlin 1924). Although P. discus has devoured up to 30% of the alfalfa weevil eggs in some locations in Europe, it has yet to become established in the United States.

CHAPTER II

DISTRIBUTION AND EFFECTIVENESS OF *BATHYPLECTES CURCULIONIS* IN OKLAHOMA

B. curculionis was first reported by D. Arnold, Survey Entomologist, Department of Entomology, in Craig County (eastern Oklahoma) in 1971. The following year it was recovered from four counties in the northwestern panhandle area and from three additional counties in northeastern Oklahoma (Figure 1). Because *B. curculionis* has not been released in Oklahoma and was first detected in two widely separated regions of the state, it is probable that it entered Oklahoma with both the eastern and western strains of the weevil (Berberet and Flora 1973).

During the spring, overwintering *B. curculionis* begin to emerge about four weeks after peak abundance of weevil larvae and parasitize first and second instar weevil larvae primarily. The parasitoid larvae feed internally and form cocoons in the cocoons spun by the host larvae (Brunson and Coles 1968). In Oklahoma, about 5% of the first generation larvae pupate and become adults (laboratory rearing of field collected parasitoids) which parasitize weevils and produce a second generation. The entire second generation and the remaining portion of the first then diapause as larvae within their cocoons until the following season (Brunson and Coles 1968).

This study was initiated to monitor the distribution of *B. curculionis* in Oklahoma and to determine its effectiveness as a biological control agent of alfalfa weevil populations in the state. The success of B. curculionis in Oklahoma is of special concern because mixing the eastern and western strains of the weevil may have an impact on parasitism of H. postica by B. curculionis in the west. If increased capacity to encapsulate parasitoid eggs is transferred through crosses of the two strains, the result could be reduced effectiveness of B. curculionis. In conjunction with distribution studies, an investigation of the rate of encapsulation of B. curculionis eggs by weevil larvae and its resulting impact on the effectiveness of this parasitoid as a control agent was initiated.

Methods and Materials

It was advantageous to divide Oklahoma into several regions for this study (Figure 1) (these regions are designated NW=northwest; C=central; NE=northeast; and SW=southwest). The primary reason for this division was to provide designated areas of reference for studies on parasitism by B. curculionis in Oklahoma. In 1972 B. curculionis occupied two widely separated areas of Oklahoma; the northeastern (NE) and northwestern (NW) corners of the state which were separated by a central section (C) from which B. curculionis was not collected. In 1973 the parasitoid was detected in low numbers in the central (C) and southwest (SW) regions of the state. The southeast region (except for Bryan, Choctaw, and McCurtain counties), having very little alfalfa acreage, was not extensively sampled during this study.

The development of weevil populations occurs at different rates during the spring in these regions due to differing climatic conditions. The buildup of weevil populations begins in the SW first, then the central region, NE region, and finally the NW region. Climatic factors affected the timing of survey trips and rendered less critical the limitations involved in attempting to sample at once all regions of the state. Division of the state into regions presented defined areas of collection within the limits of time and manpower for each survey trip.

The sampling for B. curculionis in 1972 was limited to surveys for the parasitoid because its presence in the state had been indicated in only one county in 1971. These surveys were concentrated primarily in the NW and NE, where the parasitoid would most likely be detected in areas inhabited by the weevil for a longer time. During 1973 the major emphasis was given to the NE and NW for the purpose of estimating rates of parasitism in areas B. curculionis was known to have occupied in 1972. The C and SW regions were sampled in 1973 to follow movement of the parasitoid throughout the state. Emphasis in 1974 was given to all regions (except SE) for determining the distribution of and rate of parasitism by B. curculionis throughout the alfalfa growing regions of Oklahoma. For each collection date, sampling was limited to one field per county.

Rates of parasitism computed for only one sampling date during a season can, due to fluctuations in host density, result in misleading conclusions as to the effectiveness of a parasitoid in controlling its host. For example, a late season sample (May) in Oklahoma, when weevil larval populations are declining and B. curculionis populations are increasing, would result in a high rate-of-parasitism value. This value would reflect only the late season host-parasitoid relationship when weevil damage is low, rather than the relationship during the critical period (March-April) when weevil numbers are high and control is essential. Survey trips were planned to coincide with peak larval populations. This is the period when damage is high and the highest level of parasitoid activity is desired. Late season surveys were conducted to determine the rate of parasitism in a rapidly declining host population. Because surveys were handled in this manner, the peak and late season sampling dates for 1973 and 1974 are related to weevil infestations rather than calendar dates. Generally, in Oklahoma peak larval numbers occur between March 15 and April 15 depending on weather conditions and location within the state. Late season (low population level) numbers occur between April 15 and June 1, again depending upon weather and location.

Distribution Study

Sweepnets were employed for sampling in 1972 and 1973 because the distribution of _B. curculionis_ in Oklahoma was largely unknown, and the sampling objective was to collect large numbers of weevil larvae in order to detect even very low numbers of _B. curculionis_. This sampling method features large sample sizes, which may provide more reliable data, and the advantage of collecting larger (third and fourth instar) larvae from the upper portions of the plant. These larvae, because they are more exposed, may have an increased chance of being parasitized by _B. curculionis_ and can be reared for parasitoid retrieval more rapidly and with much less mortality than can smaller larvae.

The weevil larvae collected from each field were placed in paper bags with fresh alfalfa foliage and transported to the laboratory for parasitoid retrieval. All bags containing larvae designated for retrieval were held at room temperature inside screen wire cages. The top half of each paper bag was cut away to allow for proper ventilation, and fresh alfalfa was added daily until development of larvae was completed. All foliage utilized as feed was grown in an outdoor screen cage which prevented weevil infestation. Weevil pupae and/or parasitoid cocoons in each bag were counted and their numbers recorded along with the respective county and sampling date. Retrieval samples averaged between 500 and 1000 weevil larvae per bag. From this information, the rate of parasitism for each county was calculated.

In 1974 a stem-pull method was initiated for peak season surveys which would allow collection of all larval instars from all portions of the plants. This type of collection cannot be obtained by use of a sweepnet. It was hoped that the stem-pull method would permit a much more accurate estimate of weevil and parasitoid populations. Two hundred stems were pulled per field and placed in paper bags for transport to the laboratory. A visual estimate of the plant stand of the alfalfa field was made (e.g. good=30 or more stems per square-foot; average=20-30 stems per square-foot; and poor=10-20 stems per square-foot). The number of weevil larvae and/or parasitoid cocoons collected per square foot could then be computed. However, problems with the stem-pull method forced a return to sweepnet sampling during peak season in 1974. I found that very small instars of weevil larvae were often feeding deep inside the alfalfa terminals; these terminals dried rapidly during the rearing process and trapped the small larvae inside the curled terminal leaves. This impairment effectively starved the larvae because they were not able to eat through the dried plant material. This morality factor would have decreased the accuracy of the values for parasitism if the stem-pull method had been continued throughout the sampling process. Consequently, many peak season and all late season samples were collected with sweepnets during 1974.

Encapsulation Study

Dissection samples were collected from counties where B. curculionis had been previously detected in retrieval samples. The weevil larvae designated for dissection (encapsulation study) were collected in the same manner as those for retrieval, transported to the laboratory in ice chests, and refrigerated at 40°F. Larvae were refrigerated in order to retard parasitoid development so that when dissected, they would be in about the same stage of development as when collected. Dissection of larvae was performed within 24-72 hours of their arrival in the laboratory. Except in cases of very light infestation, where sufficient numbers were not collected, 100 larvae were randomly selected and dissected from each field sampled. In fields where collections resulted in low numbers of larvae for study, the dissection method rather than the retrieval method was utilized to more clearly reveal the conditions of parasitoids.

All weevil larvae were dissected under water in glass dishes. A stereomicroscope (approximately 20X) with transmitted light was used to aid in the detection of parasitoid eggs and larvae. The number and condition (encapsulated versus healthy) of B. curculionis eggs and larvae detected were recorded, and this data was utilized to compute actual (percent weevils parasitized) and effective (percent weevils which contained healthy parasitoids) parasitism as well as the rate of encapsulation. Actual parasitism was calculated as:

AP = (a/a+c) X 100 where, a = the number of larvae containing parasitoids. c = the number of unparasitized weevil larvae. (1)

Effective parasitism was calculated as:

EP = (b/b+d) X 100 where, b = the number of weevil larvae that contain a healthy parasitoid. d = the number of unparasitized weevil larvae plus those with only encapsulated parasitoids. (2)

Results and Discussion

Since the first report of B. curculionis in Craig County in 1971, the parasitoid has rapidly expanded its range across Oklahoma. In 1972 this parasitoid was recovered from the NW and NE in 7 of the 26 NW, C, and NE counties that were sampled (Figure 1). B. curculionis entered the state as a parasitoid of two separate alfalfa weevil populations, and the spread of the parasitoid has been from the NE and NW corners of the state toward central and southern Oklahoma. Not until 1973 were parasitoids collected in adjacent counties in northern Oklahoma (Figure 1). The recorded distribution of B. curculionis in Oklahoma was expanded from 7 to 44 counties in 1973 and from 44 to 57 counties in 1974 (Figures 1, 2). These data indicate that in Oklahoma establishment of B. curculionis has occurred approximately three years after the alfalfa weevil has become established.

Not all Oklahoma counties were sampled in this study (Figures 1, 2). Oklahoma County (central) is almost exclusively urban Oklahoma City and/or its surburbs. Extreme eastern (i.e., Adair and Cherokee counties) and southeastern (i.e., Leflore and Pushmataha Counties) regions of

Oklahoma are mountainous and possess little or no alfalfa acreage. Due to intensive insecticide applications statewide for weevil control during the years this study was conducted, some counties, particularly those with small acreages (i.e., McIntosh, Okmulgee, Pawnee, Sequoyah Counties), at times contained no fields with weevil populations that could be sampled.

The dissection method of parasitoid detection was incorporated in this study to more clearly reveal the influence of encapsulation on effective parasitism. The retrieval procedure cannot provide observations as to the number and/or condition of B. curculionis eggs and/or larvae within weevil larvae. The retrieval method is adapted primarily for parasitoid detection and approximation of the rate of parasitism, while the dissection method clearly reveals actual parasitism and the extent of encapsulation of parasitoid eggs and/or larvae. In general, the yearly values for mean parasitism obtained from the two methods were in agreement for each region of the state except the NW in 1973 and the SW in 1974 (Table 1). This may be a lack of consistency between the two methods due to unequal numbers of retrieval and dissection samples from the same region. Often, in counties where B. curculionis had not been previously detected, only retrieval samples were taken, because in such counties parasitoid detection was the primary objective. This was the case in the SE in 1974 when only 7 dissection samples are compared with 18 retrieval samples.

The rates of parasitism in individual counties are useful in analyzing the weevil-parasitoid relationships on a seasonal basis where the spread and increase of B. curculionis can be followed on a county-by-county basis for the two year period. Presentation of data for actual and effective parasitism from dissection samples and parasitism from retrieval samples for 1972-74 peak and late season surveys provides a detailed overview of the distribution and impact of B. curulionis in Oklahoma (figures 3, 4, 5, 6). In most cases the rate of parasitism for a county, determined by both the dissection and retrieval methods, has shown a steady increase from peak season 1973 through late season 1974, indicating increased effectiveness of B. curculionis subsequent to establishment.

The extent to which encapsulation affected successful parasitism by B. curculionis is clearly revealed by listing of the conditions commonly observed in parasitoids dissected from weevil larvae (Tables II-V). Healthy eggs or larvae were free of haemocyte capsules and were continuing embryonic or larval development; while encapsulated eggs or larvae were enclosed in a haemocyte capsule and often exhibited a darkened and shrunken appearance which indicated death of the embryo. Dissection disclosed examples of simple parasitism such as one healthy egg (1HE); one encapsulated egg (1EE); one encapsulated larva (1EL); and one healthy larva (1HL). I observed a variety of parasitoid egg and larval combinations due to superparasitism in late season. For example, two healthy larvae plus one encapsulated egg in a host (2HL + 1EE); one healthy egg plus one healthy larva (1HE + 1HL); and three healthy eggs (3HE) were commonly observed. Less common examples of superparasitism such as one healthy larva plus one encapsulated egg plus one encapsulated larva in a host (1HL + 1EE + 1EL) or one healthy larva plus one encapsulated larva plus two encapsulated eggs in a host (1HL + 1EL + 2EE), were placed in a column entitled OTHER.

In most cases, encapsulated larvae were newly hatched, only partially surrounded by a haemocyte capsule, and apparently healthy. I feel that the partial encapsulation of such larvae was due to the accumulation of haemocytes comprising a partial capsule around an egg which then remained attached to the parasitoid larva upon hatching. In cases where dead encapsulated larvae were observed, healthy parasitoid larvae were also present. Van den Bosch (1964) has reported similar observations and suggests that dead larvae become fully encapsulated after having been killed in battle with the other parasitoid larvae present.

I was interested in the effect of supernumary eggs (superparasitism) on the encapsulation process. Puttler (1967) had noted that superparasitism reduced the impact of encapsulation on parasitism in the eastern strain of the alfalfa weevil. Thus values for the rate of encapsulation of single eggs or larvae (simple parasitism) and values for the rate of encapsulation of multiple eggs or larvae were calculated for each county in Tables II-V. A comparison of this data may enhance analysis of the relationship (if any) between superparasitism and encapsulation. The total affect of encapsulation on effectiveness of B. curculionis (regardless of number or condition of parasitoids within hosts) was calculated as the percentage of all parasitoid eggs and/or larvae encapsulated (% Par Encap) in each county sampled (Tables II-V). The means of these values for each area were calculated to aid in a discussion of the affect of encapsulation on parasitism by B. curculionis (Table VI).

I observed that simple parasitism was most prevalent in peak weevil population samples (March-April) when the ratio of weevils to parasitoids was high (Tables II and IV). Superparasitism occurred almost exclusively in late season samples when the weevil population was sharply declining (Tables III and V).

The effectiveness of *B. curculionis* was reduced in all regions of Oklahoma due to encapsulation of parasitoid eggs and/or larvae by *H. postica* larvae. The difference between actual and effective parasitism values calculated from the dissection data (Tables II-V) is due wholly to the encapsulation process. During peak season 1974, encapsulation by weevil larvae reduced actual parasitism by 4/5 in the SW and by almost 1/2 in the NW and NE (Table I).

The rate of encapsulation by weevil larvae is inversely related to the rate of parasitism by *B. curculionis* in Oklahoma. More specifically, when the rate of parasitism is low (in peak season), the rate of encapsulation is high; and when the rate of parasitism is high (in late season), the rate of encapsulation is lower. This would not be unusual if superparasitism (supernumary eggs) was so extensive in late season as to reduce the effect of encapsulation, but this is not entirely the case when mean values for the rate of encapsulation of single eggs also decline in late season samples. I could detect no apparent reason for this relationship. It may be that it is related to a physiological condition of the weevil that is influenced by weather, photoperiod, and/or diet of the weevil larvae rather than being related to the rate of parasitism. This relationship was common to all four regions of Oklahoma, and contributed to an important decrease in the effect of peak season parasitism by *B. curculionis* when the full impact of actual parasitism is most needed.

In peak season when simple parasitism was most common and superparasitism was rare (Tables II and IV), the rate of encapsulation of supernumerary eggs and/or larvae (% Super Eggs Encap) was relatively high (Table VI). But in late season when superparasitism was common, the percentage of supernumerary eggs encapsulated declined to a value much lower than the peak season value. The apparent significance of this relationship is that superparasitism, when commonly expressed in the weevil population, can reduce the effects of encapsulation by overcoming the encapsulating capacity of weevils with sheer numbers of eggs (Tables III and V). This conclusion is supported by Puttler (1967). The effect of a higher level of parasitism in the late season in reducing weevil population potential for subsequent years may be helpful in preventing weevil outbreaks. Also, late season parasitism contributes to the overwintering parasitoid population for the following spring.

In general, trends of parasitism and encapsulation were the same across Oklahoma. However, the regions sampled in this study differed as to the distribution and rate of parasitism and encapsulation of _B. curculionis_. A region-by-region discussion on the distribution and effectiveness of _B. curculionis_ is helpful in analyzing its establishment and potential parasitism in the state.

Northeastern Oklahoma

In the 1972 late season survey in NE Oklahoma, county records were reported for _B. curculionis_ in Craig (also in 1971), Ottawa, Mayes, and Tulsa counties, while no parasitoids were detected in Washington and Nowata counties. _B. curculionis_ appears to have been established only in scattered locations in the NE region and present in small numbers. In 1973, the parasitoid was detected in all 12 NE counties sampled. During this time, peak season parasitism occurred at very low rates, indicating that the B. curculionis population level was low in the counties sampled. In late season 1973 samples, parasitism increased greatly in Craig and Mayes counties, while most other counties maintained low rates of parasitism (Figure 4). The fact that B. curculionis was detected in several NE counties at very low rates of parasitism in 1973 indicates that the parasitoid became established in the NE region in 1971 or 1972 and increased to detectable numbers in 1973.

Peak season parasitism in 1974 was much higher than that in 1973. Tulsa, Rogers, Nowata, and Craig counties exhibited peak season actual parasitism rates that were high enough to eliminate substantial numbers of weevils, and the late season weevil population in NE Oklahoma was heavily parasitized with rates of parasitism ranging as high as 93% in Muskogee County (Figure 6). Although B. curculionis is well established in the NE and parasitism is increasing in both peak and late season, the rates are not yet high enough for effective biological control to occur during periods of heavy damage.

The rate of encapsulation of B. curculionis eggs in NE Oklahoma occurs at values typical of the eastern strain of the alfalfa weevil (Puttler 1967). Although the yearly average rate of encapsulation for all parasitoid eggs and larvae remained approximately the same for 1973 and 1974, the peak season percentage of parasitoids encapsulated nearly doubled in 1974 (Table VI, % PE). If this trend continues, the effect on biological control potential may be disasterous. For instance, a 48% rate of encapsulation for all parasitoid eggs and larvae (NE 1974) in peak season reduced the potential peak season parasitism from 9.3% to an average of 5.1% effective parasitism, which is a substantial reduction in larvae destroyed by parasitoids.

Northwestern Oklahoma

In the panhandle counties of Oklahoma B. curculionis exhibited moderate rates of parasitism in the 1972 survey, while it was not detected in the five other NW counties sampled (Figure 1). The peak season 1973 values for parasitism are very low in Ellis, Woodward, Dewey, and Alfalfa counties with no parasitoids detected in Blaine and Major counties (Figure 3). The 1973 peak season rates of parasitism increase in a northwesterly direction into the panhandle where parasitism is highest. Late season values for parasitism, as in the NE region, increase to values much larger than in peak season. This data indicates that B. curculionis was present in the panhandle area in detectable numbers in 1971, and was probably established in most other NW counties in 1972. It appears that B. curculionis entered NW Oklahoma from the panhandle area after being established there on western weevils since at least 1970, and then moved rapidly in a southeasterly direction into the state. B. curculionis was detected in Blaine county in late season 1973, and the 0.1% rate of parasitism indicates an established parasitoid population (Figure 4).

The 1974 peak season values for parasitism exceeded by two or three times the 1973 values at peak season (Figures 3 and 5). Similarly, the late season values also exceeded most of the 1973 late season values (Figures 4 and 6) averaging about 68% parasitism for the region (Table I). The averages for all rates of parasitism in the NW were higher than corresponding rates of parasitism in the NE for both 1973 and 1974 (Table I).

The rate of encapsulation of B. curculionis eggs in NW Oklahoma exhibited a trend similar to that noted in the NE (Table VI). While the peak season rates of encapsulation were somewhat lower in the NW than in the NE, the yearly average rates of encapsulation were higher in the NW due to higher late season rates of encapsulation. Considering such high rates of encapsulation (43% in peak season 1974), it is obvious that a very low proportion of western weevils exist in NW Oklahoma if the encapsulation criteria cited by van den Bosch (1964) and Puttler (1967) are utilized to differentiate strains. Because the western strain of the weevil occupied NW Oklahoma before 1969 and the eastern strain has consistently spread westward across Oklahoma since 1968, the majority of weevils now occupying NW Oklahoma must be either eastern strain or eastern-western hybrids with few western weevils present. The encapsulation data from the NW indicates that the greater ability of the eastern strain to encapsulate B. curculionis eggs and/or larvae is transferred to the eastern-western hybrid offspring in a cross with western strain weevils.

Central Oklahoma

Twelve central counties were sampled for B. curculionis in the 1972 survey with negative results. Grant, Garfield, and Payne counties had low numbers of parasitoids in peak season 1973. Seven other counties in south-central Oklahoma yielded no parasitoids. However, 14 new county records were added in late season 1974 and 8 new records were added in 1974 (Figures 1 and 2). The parasitoid was first detected in the northern and eastern portions of the central region and exhibited relatively high rates of parasitism in these areas. I believe the parasitoid spread into central Oklahoma primarily from the NE region. I also feel that our sampling coincided with the spread of B. curculionis into most of central Oklahoma, because several samples were often taken before the parasitoid was detected. For instance, B. curculionis was not detected in some west-central counties until 1974 although they were sampled in late season 1973 (Figures 4 and 6). Logan County was sampled four times throughout the 1973 season, and only in very late season was the parasitoid detected.

Peak season rates of parasitism averaged almost 0% in 1973, but showed a definite increase in 1974 (4%) due to the spread of B. curculionis into additional counties during the late season 1973 and in 1974. The parasitoid rapidly increased its distribution and population levels in central Oklahoma averaging almost 50% actual parasitism in late season 1974 (Table I). This level of parasitism approaches the rates of parasitism exhibited in NW and NE Oklahoma for the same time period; however, in central Oklahoma the large increase occurred only one year after detection in most central Oklahoma counties. The most probable explanation for the rapid increase in parasitism is that central Oklahoma is the region of concentrated alfalfa acreage in the state, while the NW and NE regions contain widely separated fields of alfalfa. It is much easier for a parasitoid to disseminate to alfalfa fields which are separated only by short distances as in central Oklahoma. The rapid establishment of B. curculionis is encouraging because this is the primary alfalfa growing area of the state, and has experienced severe problems with the alfalfa weevil.

For the first year of establishment, the rate of encapsulation of parasitoid eggs in C Oklahoma averaged 28% in peak season samples during

1974 (Table VI, % PE). Although this rate of peak season encapsulation is a lower value than corresponding rates in the NE and NW during 1974, it is a comparatively high rate for the first year of establishment (Table VI). Overall, a yearly average rate of 19% encapsulation for 1974 reduced parasitism from 21% (actual parasitism) to 18% (effective parasitism).

Southwestern Oklahoma

Sampling was begun in SW Oklahoma in late season 1973. At a time when parasitism in established populations is very high, four counties exhibited rates of only 2% or less, and samples in Greer and Kiowa counties yielded no B. curculionis. I feel that, as in central Oklahoma, the sampling coincided with the initial spread of the parasitoid into the SW region. The large increase in late season parasitism in 1974 in Roger Mills and Beckham counties was similar to that noted in other regions of Oklahoma during this study. The levels of parasitism and the distribution of parasitism in the SW indicates that B. curculionis moved into this area from the NW region of Oklahoma rather than from the NE or central regions. The eastern border counties in the SW (Commanche, Cotton) exhibited small rates of parasitism even in late season 1974; indicating that the parasitoid was just beginning to populate these counties (Figure 6). In contrast, Roger Mills, Beckham, Custer, and Greer counties on the western border of the SW region exhibited low levels of parasitism a year earlier in 1973 and much higher rates late in 1974 (Figures 4 and 6). These data, in addition to the fact that no parasitoids were found in Kingfisher, Canadian, and Grady counties in 1973 (western portion of the central region), support my contention that *B. curculionis* entered the SW from the parasitoid population in NW Oklahoma in 1973.

The 1974 peak season average of 83% of the parasitoids encapsulated (Table VI) is misleading, because this average was calculated from a total of six parasitoid eggs detected in the peak season dissection samples (Table IV). The yearly average indicates that encapsulation of *B. curculionis* eggs in SW Oklahoma occurs at rates similar to those rates exhibited in the NE and C regions.

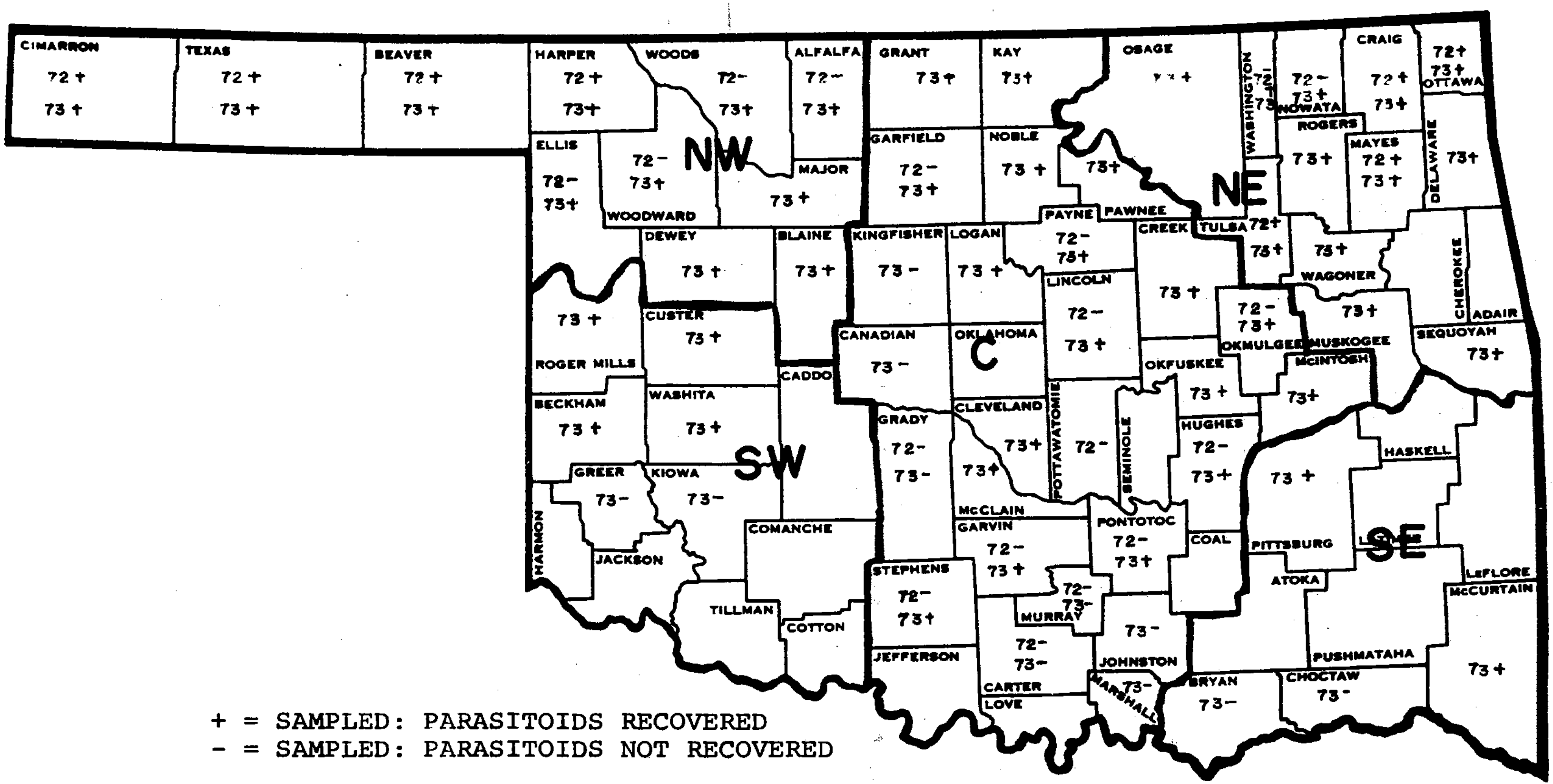

Figure 1. Sampling and Recovery of B. curculionis in Oklahoma in 1972, 1973

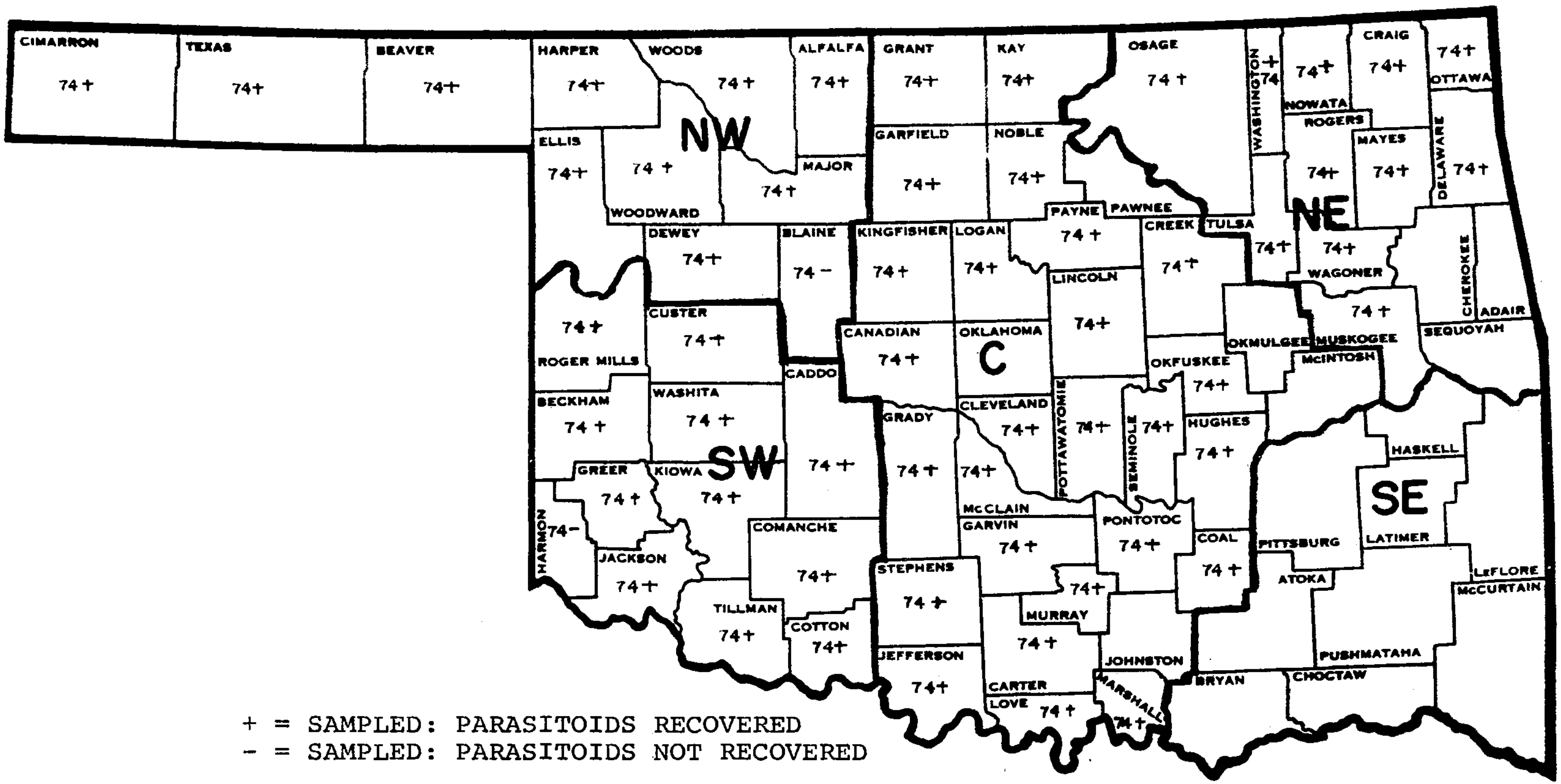

Figure 2. Sampling and Recovery of B. curculionis in Oklahoma in 1974

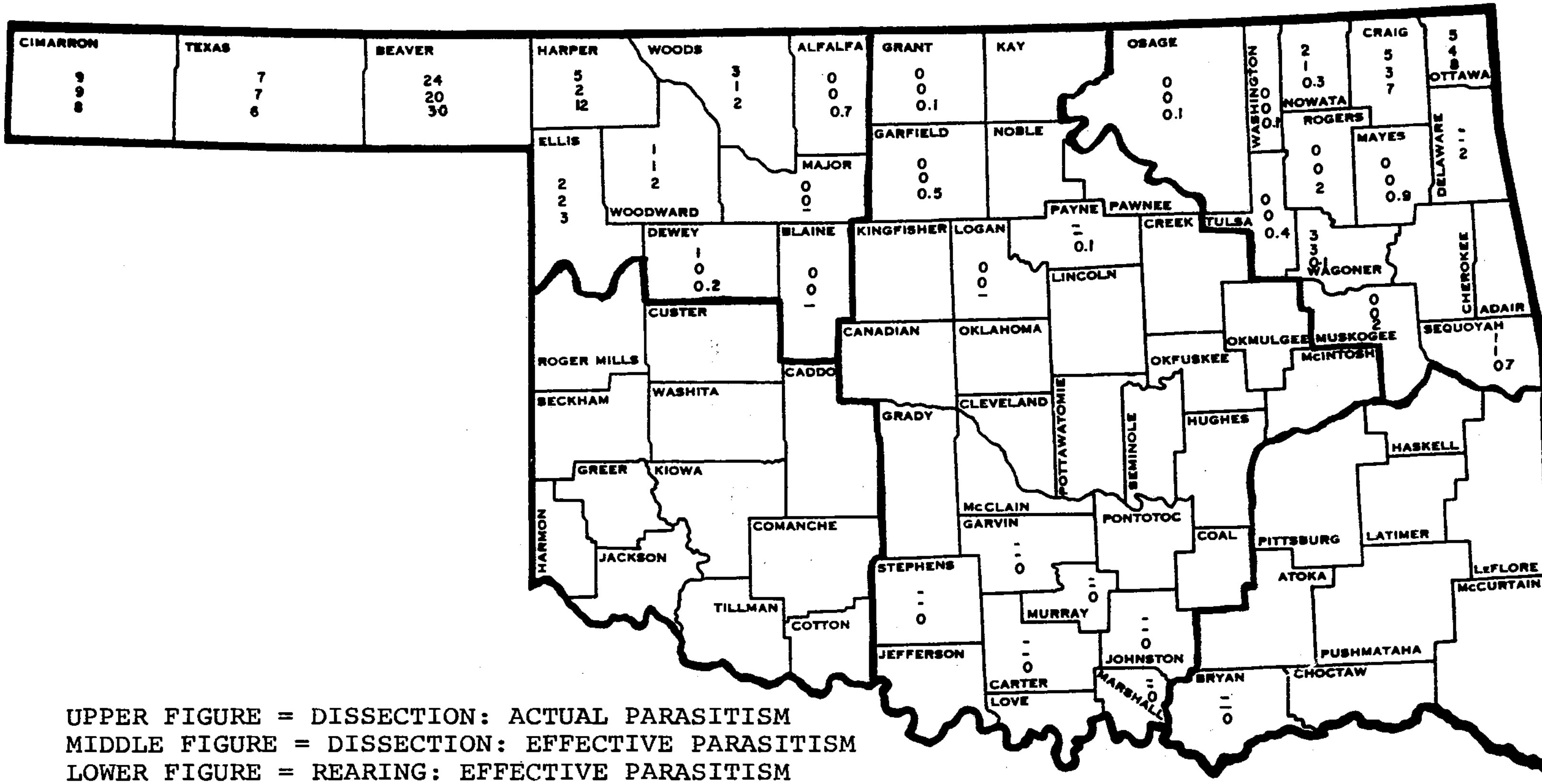

Figure 3. Parasitism of H. postica by B. curculionis, Peak Season 1973

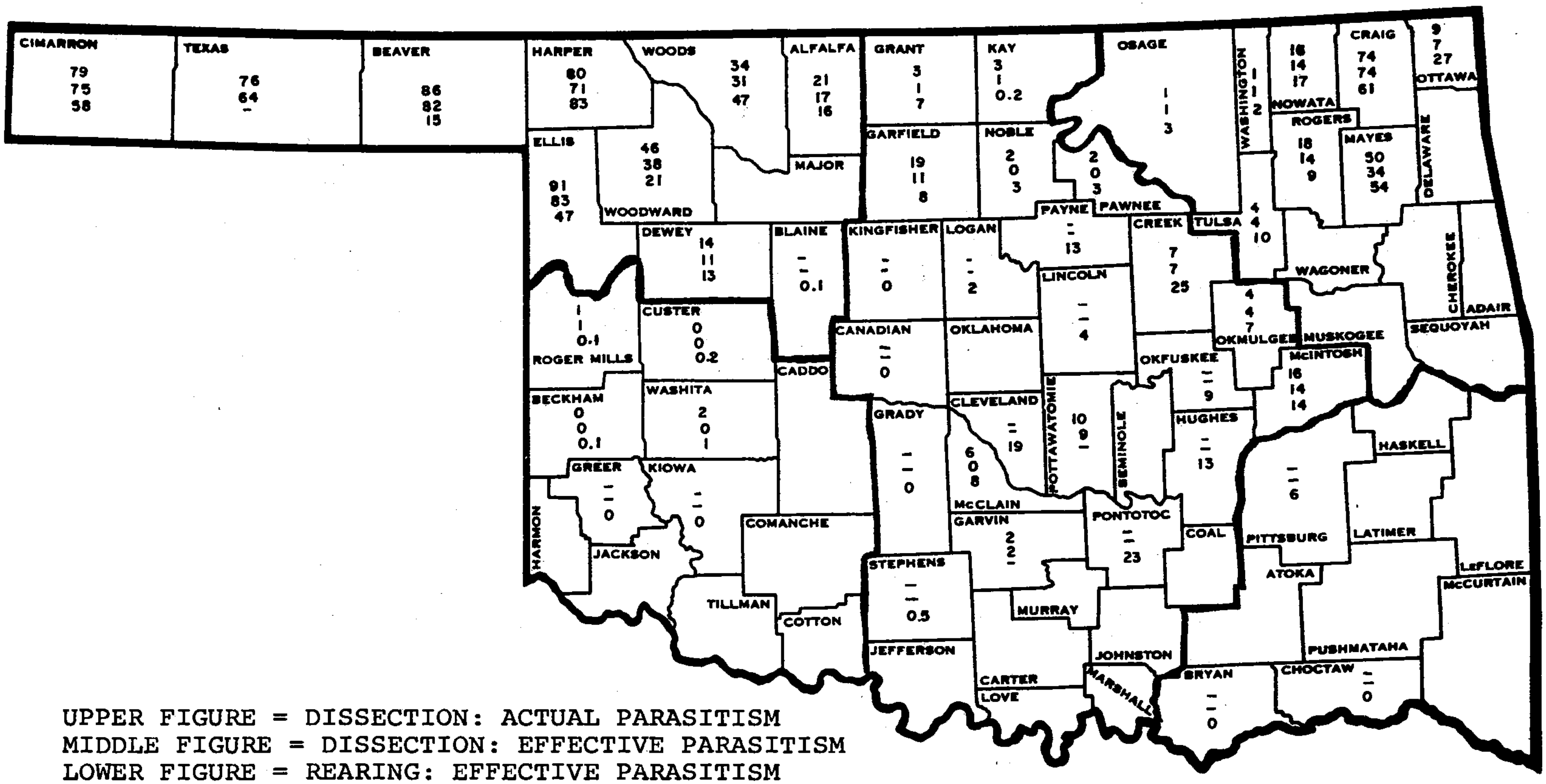

Figure 4. Parasitism of H. postica by B. curculionis, Late Season 1973

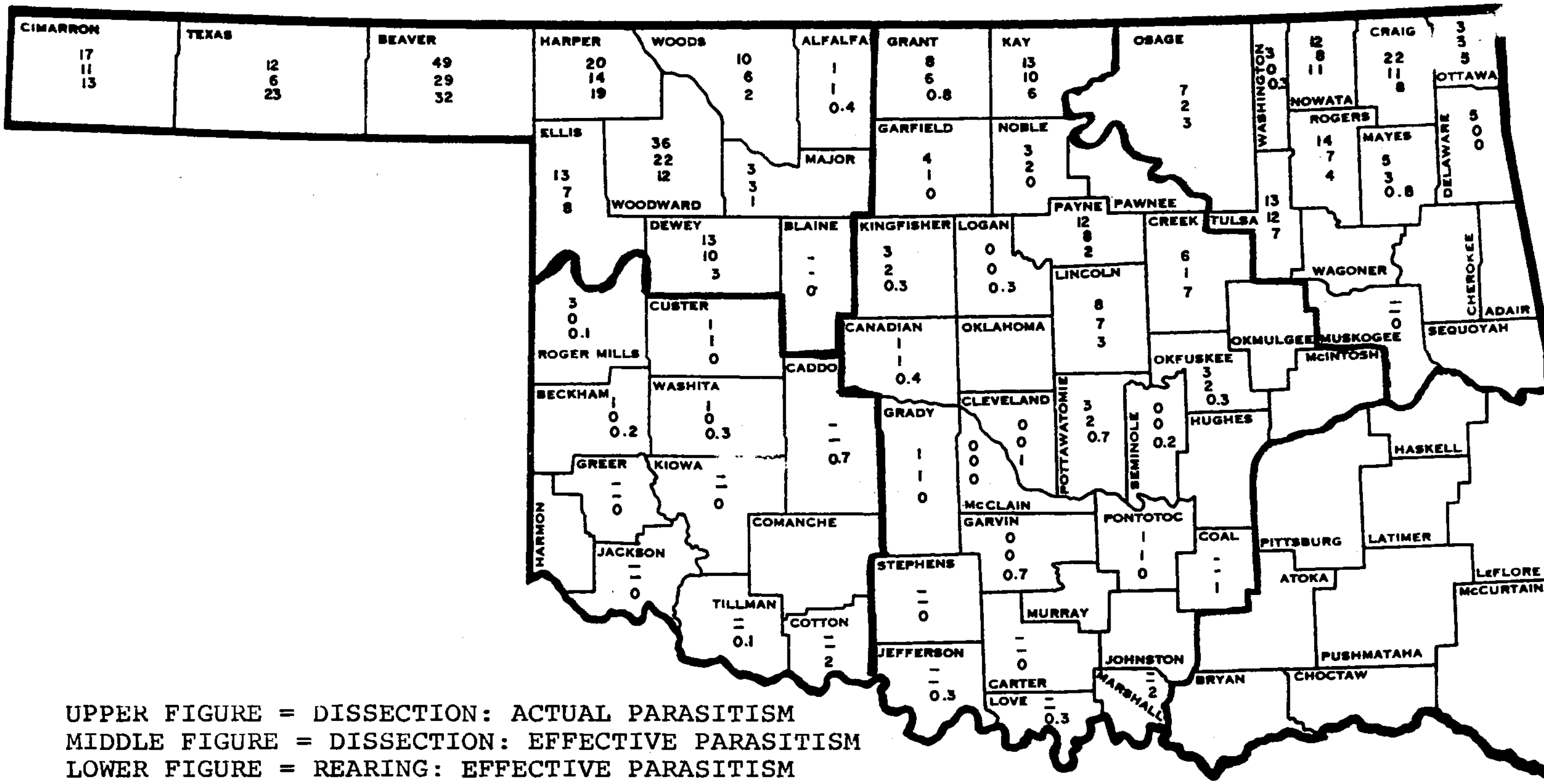

Figure 5. Parasitism of H. postica by B. curculionis, Peak Season 1974

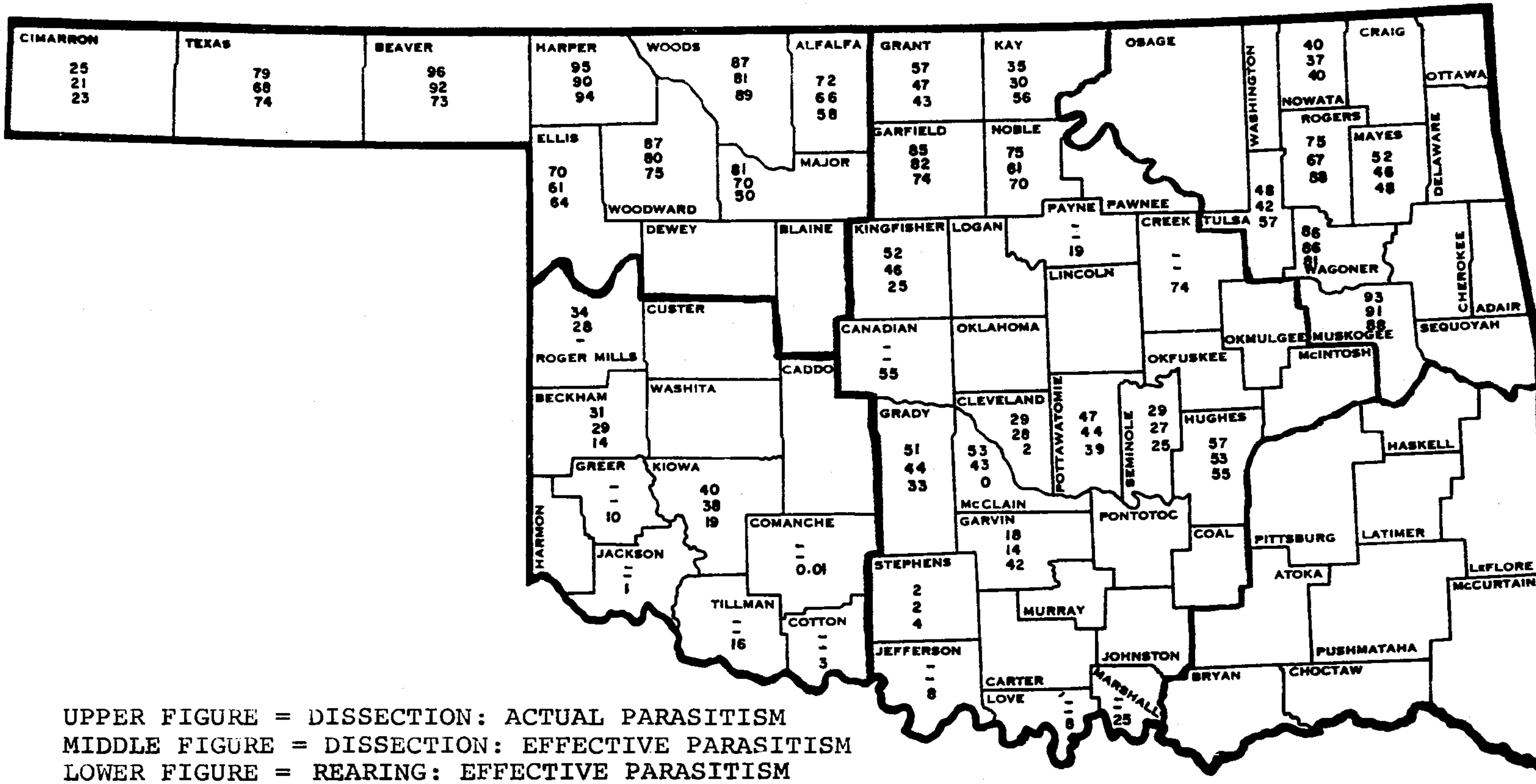

Figure 6. Parasitism of H. postica by B. curculionis, Late Season 1974

TABLE I

WEIGHTED MEANS OF ACTUAL AND EFFECTIVE PARASITISM OF
H. POSTICA BY *B. CURCULIONIS*, 1973-4

	NORTHEAST			NORTHWEST			CENTRAL			SOUTHWEST		
	DISS. Act.	DISS. Eff.	RETR. Eff.	DISS. Act.	DISS. Eff.	RETR. Eff.	DISS. Act.	DISS. Eff.	RETR. Eff.	DISS. Act.	DISS. Eff.	RETR. Eff.
1973 peak	1.4	1.1	1.7	4.7	3.8	5.9	0	0	0.1	-	-	-
late	21.6	18.6	24.7	58.5	52.4	29.8	5.8	4.1	5.9	0.6	0.3	0.2
Yr. avg.	9.9	8.5	7.6	29.0	25.7	10.1	4.3	3.1	4.1	0.8	0.3	0.2
1974 peak	9.3	5.1	5.1	19.0	11.8	11.4	3.9	2.6	2.4	1.5	0.3	0.5
late	65.7	61.5	62.7	69.2	62.9	68.9	49.0	43.3	25.7	26.8	24.3	6.4
Yr. avg.	31.9	27.7	24.8	47.9	40.8	42.8	21.3	18.3	10.4	14.1	12.3	1.2

DISS = Dissection method

Act. = Actual Parasitism

Eff. = Effective parasitism

RETR. = Retrieval Method

TABLE II

DISSECTIONS OF *H. POSTICA* LARVAE FOR DETECTION OF ENCAPSULATED EGGS OR LARVAE OF *B. CURCULIONIS* PEAK SEASON 1973

COUNTY	UNPARASITIZED	1HE	2HE	3HE	1EE	2EE	(2EE)	1HL	2HL	1EL	1HE + 1EE	1HE + 1HL	1HL + 1EE	1HL + 2EE	2HL + 1EE	1HL + (2EE)	1HL + 1EL	1EL + 1EE	1HE + 1HL + 1EE	OTHER	% EGGS ENCAP.	% SUPER EGGS ENCAP.	% PAR. ENCAP.	% ACTUAL PAR.	% EFFECT. PAR.
NORTHEAST																									
OSAGE	100																				0		0	0	0
WAGONER	97							3													0		0	3	3
WASHINGTON	100																				0		0	0	0
TULSA	100																				0		0	0	0
CRAIG	95				2			3													40		40	5	3
ROGERS	100																				0		0	0	0
NOWATA	98	1			1																50		50	2	1
MAYES	100																				0		0	0	0
OTTAWA	95				1			4													20		20	5	4
SEQUOYAH	99							1													0		0	1	1
MUSKOGEE	100																				0		0	0	0
NORTHWEST																									
TEXAS	93							7													0		0	7	7
CIMARRON	*69	1						6													0		0	9	9
BEAVER	76				4			20													17		17	24	20
HARPER	95				3			2													60		60	5	2
ELLIS	98							2													0		0	2	2
WOODWARD	99							1													0		0	1	1
WOODS	97				2			1													67		67	3	1
DEWEY	99	1																			0		0	1	1
MAJOR	100																				0		0	0	0
ALFALFA	100																				0		0	0	0
BLAINE	100																				0		0	0	0
CENTRAL																									
GRANT	100																				0		0	0	0
GARFIELD	100																				0		0	0	0
LOGAN	100																				0		0	0	0

TABLE III

DISSECTIONS OF H. POSTICA LARVAE FOR DETECTION OF ENCAPSULATED EGGS OR LARVAE OF B. CURCULIONIS, LATE SEASON 1973

COUNTY	UNPARASITIZED	1HE	2HE	3HE	1EE	2EE	(2EE)	1HL	2HL	1EL	1HE + 1EE	1HE + 1HL	1HL + 1EE	1HL + 2EE	2HL + 1EE	1HL + (2EE)	1HL + 1EL	1EL + 1EE	1HE + 1HL + 1EE	OTHER	% EGGS ENCAP.	% SUPER EGGS ENCAP.	% PAR. ENCAP.	% ACTUAL PAR.	% EFFECT, PAR.
NORTHEAST																									
OSAGE	99							1													0		0	1	1
CRAIG	26							70					4								0	50	5	74	74
TULSA	96							4													0		0	4	4
OTTAWA	91				2			6									1				25	50	33	9	7
NOWATA	84				2			13									1				13	50	18	16	14
WASHINGTON	99							1													0		0	1	1
ROGERS	82				4			13									1				24	50	26	18	14
MAYES	50				13	3		31									3				30	75	39	50	34
NORTHWEST																									
TEXAS	24	3	4	2	9		1	22	4		1	2	9	3		1			1	13	26	30	24	76	64
CIMMARON	21	5	4	8	3	1		33				4	6	2		1	9			3	7	24	19	79	75
BEAVER	14	4	2	3	3			59	3	1		7	2							2	6	9	5	86	82
HARPER	20	14			9			40	1		1		10				1			4	14	45	26	80	71
ELLIS	9	6	2		2	1	3	34	5		1	5	17	4		1	2			8	5	41	32	91	83
WOODWARD	54	5	4		6	1	1	15					9	1			2			2	23	47	42	46	38
ALFALFA	79	7			4			5					4	1							38	55	37	21	17
WOODS	20	3			2			29					3	1							6	56	14	34	31
DEWEY	86	2	1		3			6			1		1								27	33	29	14	11
CENTRAL																									
GRANT	97				2			1													66		66	3	1
GARFIELD	81	3	1		8			6					1								25	47	43	19	11
OKMULGEE	96							4													0		0	4	4
MCINTOSH	84				2			14													13		13	16	14
CREEK	93							7													0		0	7	7
PAWNEE	98				2																100		100	2	0
KAY	97				2			1													67		67	3	1
NOBLE	98				2																100		100	2	0
POTTAWATOMIE	*52				1			4					1								20	50	29	10	9
MCCLAIN	94				5					1											100		100	6	0
GARVIN	98							2													0		0	2	2
SOUTHWEST																									
WASHITA	98				2																100		100	2	0
ROGER MILLS	99							1													0		0	1	1
CUSTER	100																				0		0	0	0
BECKHAM	100																				0		0	0	0

TABLE IV

DISSECTIONS OF *H. POSTICA* LARVAE FOR DETECTION OF ENCAPSULATED EGGS OR LARVAE OF *B. CURCULIONIS*, PEAK SEASON 1974

COUNTY	UNPARASITIZED	1HE	2HE	3HE	1EE	2EE	(2EE)	1HL	2HL	1EL	1HE + 1EE	1HE + 1HL	1HL + 1EE	1HL + 2EE	2HL + 1EE	1HL + (2EE)	1HL + 1EL	1EL + 1EE	1HE + 1HL + 1EE	OTHER	% EGGS ENCAP.	% SUPER EGGS ENCAP.	% PAR. ENCAP.	% ACTUAL PAR.	% EFFECT. PAR.
NORTHEAST																									
OSAGE	93				5			1					1								83	50	75	7	2
CRAIG	78	6			11			4					1								52	50	52	22	11
TULSA	87	2			1			8					2								9	50	20	13	12
OTTAWA	97							2					1								0	50	25	3	3
NOWATA	88	1			4			6					1								36	50	38	12	8
WASHINGTON	97				3																100		100	3	0
ROGERS	86	5			7			2													50		50	14	7
MAYES	95	2			1	1		1													50	100	50	5	3
DELAWARE	*37				2																100		100	5	0
NORTHWEST																									
TEXAS	88	1			6			5													50		50	12	6
CIMARRON	83	2			6			10					2								40	50	42	17	11
BEAVER	51	2			16	2	1	24		1	1		2								40	75	47	49	29
HARPER	80	2			6			10					2								33	50	36	20	14
ELLIS	87	3			6			1					3								60	50	56	13	7
WOODWARD	64	1			14			18				1	2								42	33	41	36	22
ALFALFA	99							1													0		0	1	1
WOODS	90				4			5					1								44	50	45	10	6
DEWEY	87	2			3			8													23		23	13	10
MAJOR	*69	1						1													0		0	3	3
CENTRAL																									
GRANT	92	3			2			3													25		25	8	6
GARFIELD	96				3			1													75		75	4	1
OKFUSKEE	97	2			1																33		33	3	2
CREEK	94				5			1													83		83	6	1
KAY	87	1			3			8													25	50	29	13	10
NOBLE	97				1			2													33		33	3	2
POTTAWATOMIE	97	1			1			1													33		33	3	2
MCCLAIN	100																				0		0	0	0
GARVIN	100																				0		0	0	0
SEMINOLE	100																				0		0	0	0
GRADY	99				1																100		100	1	0
LOGAN	*55																				0		0	0	0
CANADIAN	99												1								0	50	50	1	1
PAYNE	88	5			4			3													33		33	12	8
LINCOLN	92	2			1			5													13		13	8	7
HUGHES	95	1						4													0		0	5	5
PONTOTOC	99							1													0		0	1	1
CLEVELAND	100																				0		0	0	0
SOUTHWEST																									
WASHITA	99				1																100		100	1	0
ROGER MILLS	97				3																100		100	3	0
CUSTER	99							1													0		0	1	1
BECKHAM	99							1													0		0	1	1

TABLE V

DISSECTIONS OF H. POSTICA LARVAE FOR DETECTION OF ENCAPSULATED EGGS OR LARVAE OF B. CURCULIONIS, LATE SEASON 1974

COUNTY	UNPARASITIZED	1HE	2HE	3HE	1EE	2EE	(2EE)	1HL	2HL	1EL	1HE + 1EE	1HE + 1HL	1HL + 1EE	1HL + 2EE	2HL + 1EE	1HL + (2EE)	1HL + 1EL	1EL + 1EE	1HE + 1HL + 1EE	OTHER	% EGGS ENCAP.	% SUPER EGGS ENCAP.	% PAR. ENCAP.	% ACTUAL PAR.	% EFFECT. PAR.
NORTHEAST																									
TULSA	52	8			5	1		29	1		1	2								1	12	31	17	48	42
NOWATA	60	1			3			33	1				2								12	33	13	40	37
ROGERS	25	4	4		5		1	44	2	1		2	5		1		3	1		2	11	29	18	75	67
MAYES	48	6	5		2			27		3		2	4				1		1	1	13	28	17	52	46
MUSKOGEE	7	1	1		2			65				7	15							1	3	32	16	93	91
WAGONER	14	6	1					70	3			5	1								0	5	1	86	86
NORTHWEST																									
TEXAS	21	9			5	4		40				1	7	4			3	1		5	9	60	37	79	68
CIMARRON	75	8	2		4			7					3	1							21	38	28	25	21
BEAVER	4	1			1		2	53	1			1	18	2		1	5		1	8	2	56	38	96	92
HARPER	5				4			57	2	1		2	14	3		1	4		3	4	7	45	28	95	90
ELLIS	30	9			7	1		46		1			3				1			2	13	44	18	70	61
WOODWARD	13	1	1		7			62	2			2	7				3		2		10	33	18	87	80
ALFALFA	28	8		1	6			45	1			6	4	1							10	21	14	72	66
WOODS	13	4	1	2	5		1	63	1			2	7							1	7	27	13	87	81
DEWEY																									
MAJOR	19	6	2		10	1		42	1		1	5	7	2			3			1	17	48	27	81	70
CENTRAL																									
GRANT	43	1			9			38				2	3			1		1		3	19	44	26	57	47
GARFIELD	15	1	1	2	2			51	3	1		1	16	1	1					5	19	42	23	85	82
KAY	65	5			5			20			1	2	2								17	33	7	35	30
NOBLE	25	2		1	13	1		34	3		1	6	5		1				2	5	27	22	24	75	61
POTTAWATOMIE	53	5	2	1	3			27	2			3	2						1	1	9	11	10	47	44
MCCLAIN	40	6	1	1	9			13	2			4	2							5	32	7	16	53	43
GARVIN	82	6		1	4			7													24	0	20	18	14
SEMINOLE	71	4			2			15	1		1		6								10	44	18	29	27
GRADY	49	11		1	7			19	3		2	3	2		1					2	18	16	17	51	44
HUGHES	43	6	1		4			33	1		1	5	4			1				1	9	23	15	57	53
CLEVELAND	71	4	2	1				17	1	1			1							2	5	6	5	29	28
KINGFISHER	48	7	1		5	1		36				1	1								10	50	14	52	46
STEPHENS	98							1				1									0	50	33	2	2
SOUTHWEST																									
ROGER MILLS	*53	3			5			18				1									19	0	18	34	28
BECKHAM	69	4			2			25													6		6	31	29
KIOWA	60	4			2			30					2				2				50	6	14	40	38

TABLE VI

MEAN VALUES OF RATE OF ENCAPSULATION OF
B. CURCULIONIS EGGS BY
H. POSTICA LARVAE

	Northeast			Northwest			Central			Southwest		
Date	%EE	%SEE	%PE	%EE	%SEE	%PE	%EE	%SEE	%PE	%EE	%SEE	%PE
1973 peak	25	-	25	18	-	18	-	-	-			
late	13	62	20	13	34	24	36	33	36			
Yr. avg.	14	-	20	14	-	24	-	-	-			
1974 peak	47	58	48	40	56	43	33	50	28	18	-	83
late	7	27	14	9	42	25	17	26	18	10	40	13
Yr. avg.	15	29	19	17	43	28	19	26	19	11	-	17

%EE = percentage of single eggs or larvae only which are encapsulated.

%SEE = percentage of supernumary eggs and/or larvae encapsulated not including single eggs.

%PE = percentage of all (single + supernumary) eggs and/or larvae encapsulated.

CHAPTER III

RELEASE AND ESTABLISHMENT OF HYMENOPTEROUS CONTROL AGENTS

The establishment of a successful biological control program greatly aids the development of an integrated control program comprised by a combination of cultural, biological and chemical control practices. B. curculionis is not expected to become an entirely effective biological agent in such a program in Oklahoma because its potential is reduced due to non-synchronization with peak weevil larva populations and by encapsulation. A biological program which relies upon several species for control of a pest is well founded because reduction or elimination of a single agent by adverse conditions will not result in failure of the program.

This investigation was initiated to attempt establishment of additional biological control agents of the alfalfa weevil in Oklahoma. It is proposed that establishment of parasitoids or predators which attack different life stages of the weevil will complement parasitism by B. curculionis with little interspecific competion.

The Introduced Beneficial Insects Laboratory, ARS, USDA, provided B. anurus, T. incertus, and P. discus for release in Oklahoma in 1972. These Hymenoptera attack first and second instar weevil larvae, third and fourth instar weevil larvae, and weevil eggs respectively. In 1973 M. aethiops, which attacks adult weevils, was released. Successful establishment of these species would lend stability and increased effectiveness to a biological control program for the alfalfa weevil in Oklahoma with little interspecific competition between the controlling agents.

Methods and Materials

To insure favorable conditions for establishment, selection of release sites was made according to the following criteria: that each release field have a large host population which would not be subjected to insecticide application for at least 3 years, and each field be located in an important alfalfa growing area of the state to insure dispersal of parasitoids. Considering these requirements, the 1972 release sites selected were near Duncan (Stephens County) on the Lyndel Strain ranch, and near Stillwater (Payne County) on the George Berry farm. Another release site was selected near Guymon (Texas County) in 1974 on the Ron White ranch.

The technique as suggested by Introduced Beneficial Insects Laboratory for release of all parasitoids and predators involved placing the parasitoid container on the ground, partially removing the lid, and bending alfalfa foliage over the container. This allowed the parasitoids, upon leaving the container, to alight on foliage and thus prevented excessive dispersal. All parasitoids were allowed to leave the carton at their own pace before the carton was removed and examined for dead parasitoids.

Larval samples for determination of weevil population density were taken weekly in the two release fields at Duncan and Stillwater during 1973-74. Five one-square-foot samples of alfalfa foliage were cut at ground level, placed in paper bags, and processed in berlese funnels to recover weevil larvae. The host population was then expressed as weevil larvae per square foot.

Releases of P. discus adults were made in the Duncan release field on March 22, 1972, and April 1, 1972. Three hundred of the predators were liberated on March 22 when the host population was recorded at 40 weevil eggs/ft^2 (determined by method developed by Pass 1966), and 377 predators were liberated on April 1 (host population not recorded). Sampling for P. discus was initiated in early February 1973. Five one-square-foot samples of alfalfa stems were collected from ground trash and stubble each week throughout the month at the Duncan release area. These stems were returned to the laboratory in plastic bags where they were split by use of a razor blade and examined under a stereoscope to locate P. discus larvae feeding upon weevil eggs inside the stems. No samples were taken for P. discus during the 1974 season.

B. anurus was released at Stillwater on April 11, 1972, (279 adults released) and again on April 30, 1972 (198 adults released). Alfalfa weevil populations in the release field were recorded at 100 larvae/ft^2 (April 11) and 14 larvae/ft^2 (April 30). At the time of release, wasps were observed parasitizing weevil larvae.

One release of 207 B. anurus adults was made near Duncan on March 17, 1972. Again parasitism by B. anurus was observed immediately after release in the field. Host density on March 17 was recorded at 350 weevils larvae/ft^2.

An additional parasitoid release field designated the "subrelease" field was established in Stephens County. On June 1, 1973, 79 diapausing cocoons, which were obtained from retrieval of B. anurus from the

Oklahoma release areas in 1973, were placed in this area. The cocoons were placed on the soil surface in alfalfa crowns to simulate natural conditions. This site offers the cocoon natural protection from intensive heat of direct sunlight, washing away in the high water from rains, and from direct exposure to pesticides and other chemicals.

On May 4, 1974, 458 B. anurus adults were released near Guymon in Texas County. The host population was estimated to be 100-200 larvae/ ft^2 on the release date.

In 1972, samples were taken 1-3 weeks after release in order to determine if successful parasitism (not establishment) had occurred upon release. Samples for B. anurus were taken once a week in 1973 and twice weekly in 1974 beginning each year as soon as weevil larvae were detected in the release fields. Establishment would be confirmed only if B. anurus was recovered one year or longer after the release dates. Recovery after one year would demonstrate that the parasitoid can successfully parasitize the weevil and complete its life cycle in Oklahoma. In the early season, when larvae could not be collected by use of a sweepnet, terminals were pulled in order to collect weevils. The samples containing larvae were bagged and marked with sampling date and location. Sampling was performed by sweeping weevil larvae from foliage in release areas during mid and late season. Depending upon the host population, a variable number of sweeps were made in an effort to obtain a substantial number of larvae (500-1000) for each sampling date. A large sample size was necessary because, if established, the parasitoids would be present in very low numbers and difficult to detect in small sized samples. Screen wire retrieval cages were utilized for rearing of larvae to retrieve weevil pupae and/or parasitoid cocoons. The top half of paper bags was cut away to allow ventilation and addition of fresh alfalfa daily until the weevil larvae had pupated.

Parasitoid retrieval involved careful examination of all foliage within the bags for parasitoid cocoons and weevil pupae. The number of parasitoid cocoons and weevil pupae from each bag was recorded for each sampling date, and the rate of parasitism was calculated from this data. The cocoons of B. anurus are easily distinguishable from those of B. curculionis by the peculiar jumping behavior exhibited by the B. anurus cocoons. Recovery of both B. anurus and B. curculionis can be accomplished by the same sampling methods. Because B. curculionis was known to be spreading into the release areas, we expected to recover both parasitoids during sampling for B. anurus. In addition, a situation in which both parasitoids were becoming established in the same area presented the opportunity to compare parasitism by the two species.

On March 27, 1972, 127 T. incertus adults were released at the site near Duncan. Host population was recorded at 300 larvae/ft^2 at the time of release. Retrieval procedures for T. incertus were the same as those described for retrieval of B. anrus. Cocoons of T. incertus can be recognized by the dark-brown mummified appearance of the dead weevil larvae containing the parasitoids. Sampling for T. incertus began at the same time as that for B. anurus and ended when larvae could not be collected in the release field (usually late May).

M. aethiops adults were released in the Duncan release field on May 31 (194 released) and June 6, 1973 (134 released). The host population was recorded as being a moderate level of weevil adults on the release dates. At the Stillwater release field, 129 M. aethiops adults were released on June 13, 1973, and 122 adults on June 25, 1973. The releases of M. aethiops on these late season dates very likely prevented the parasitoid from completing two generations during the 1973 season, because primarily diapausing (newly-emerged) adult weevils were available for attack rather than overwintered adult weevils. The population of newly emerged weevils adults was reported as light at the Stillwater field on the release dates.

Recovery procedures for M. aethiops were begun in late May 1974 when weevil adults soon to begin aestivation were present in release fields. Adults were collected at night from the Duncan and Stillwater release fields and other surrounding fields to check for possible parasitoid dispersal from the release fields to the surrounding areas. Weevil adults were collected with a sweepnet, placed in paper bags with alfalfa foliage, and transported to the laboratory. All adult weevils awaiting dissection for parasitoid detection were maintained in cartons in cold storage at 40°F and supplied with a sugar solution for food. The method of detection for M. aethiops larvae in weevil adults is performed by removal of the elytra and wings from the weevil and dissection of the abdomen. Dissections were performed under water utilizing a stereomicroscope to aid in parasitoid detection. The parasitoid, if present, will float free of the hemocoel as described by Drea (1968).

Results and Discussion

Peridesmia discus

It is known that in France P. discus requires high populations of weevil eggs in stems for survival. P. discus larvae feed only on weevil eggs. Winter weather must be mild to allow weevil and P. discus adults to become active, but not so warm that weevil eggs will hatch before

P. discus larvae can complete development. The Oklahoma winter of 1972-73 was unusually cold and wet. Consequently, few weevil adults were active during January and February, the result being low numbers of overwintering weevil eggs. Lack of food and harsh survival weather for *P. discus* very likely contributed to the failure to establish this species in the Duncan release field.

Bathyplectes anurus

In 1972, samples were collected with sweepnets at the Stephens and Payne county release sites and processed for retrieval of *B. anurus*. Three *B. anurus* cocoons were recovered from a sample of 200 weevil larvae collected on April 16, 1972, at the Payne county release site. At the Stephens county release site, 2 parasitoid cocoons were recovered from 125 weevil larvae collected on April 3, 1972, and 1 parasitoid cocoon was recovered from 285 weevil larvae collected on April 5, 1972. The recovery of *B. anurus* cocoons during the spring of 1972 indicates that the parasitoid did successfully parasitize weevil larvae upon release, however, establishment would be indicated only if *B. anurus* cocoons were recovered the following spring (1973).

Retrieval efforts for *B. anurus* were successful during 1973 and 1974 at both Oklahoma release sites in Payne and Stephens counties. A total of 524 *B. anurus* cocoons have been collected from these release sites during the two-year study. The retrieval data for 1973 (Table VII) indicates that *B. anurus* parasitized weevils from the last week in March until May in Stephens county. The highest rates of parasitism by *B. anurus* in this area, 0.8% and 0.5%, occurred in late March and early April. At the Payne County release site in 1973 (150 miles north of the

Stephens County site) B. anurus began parasitizing weevil larvae on April 12 and continued until mid-May. The highest rates of parasitism occurred in late April through the first week in May. A total of 78 B. anurus cocoons, 29 each from Stephens and Payne Counties, were recovered during the 1973 study.

Two B. curculionis cocoons were recovered in the May 17 sample from the Payne County site, and two B. curculionis cocoons were recovered in the May 18 sample at the Stephens County site. Recovery of these cocoons (both recoveries being county records) indicated that B. curculionis was increasing its range into the Payne and Stephens County areas. This provided the opportunity to study the relationship between the two species of parasitoids in Oklahoma.

In 1974 the establishment of B. anurus was confirmed with increases in parasitism at both Oklahoma release areas (Tables VIII and X). Although rates of parasitism are not high enough for economic control of weevil population, the numbers of B. anurus increased five to ten fold.

One hundred and forty three B. anurus cocoons were recovered from the Duncan area between March 12 and April 26, 1974. The highest rates of parasitism occurred during late March and early April. Although B. curculionis was present in low numbers during this same period, its peak activity came later in the season. B. anurus exhibited peak parasitism about three weeks before B. curculionis at the Stephens County site. Evidence of dissemination of B. anurus from the release area was obtained when seven (April 6) and eight (April 12) cocoons were collected from an alfalfa field across a wooded area approximately 0.2 miles west of the original release area. Successful recovery of B.

anurus from the "subrelease" area (Table IX) indicates that this parasitoid can be released in Oklahoma as diapausing larvae in cocoons collected from release fields in Oklahoma.

An indicator of the potential of B. anurus is its sychrony in peak weevil populations. Although B. curculionis showed a higher rate of parasitism than B. anurus at both Oklahoma release sites, this rate occurred three to five weeks after the peak weevil larval populations (Figures 7 and 8). The lag between peaks of host and parasitoid activity caused a decrease in the overall effect of parasitism by B. curculionis. At the Stephens County release site in 1974, the highest rate of parasitism occurred only one week after the weevil population had peaked (Figure 7). This timing approaches the synchrony strived for in successful biological control programs.

At the Payne County site in 1974, B. anurus was first recovered during the first week of April and showed a relatively steady rate of parasitism throughout April and early May. The highest rate of parasitism during peak weevil populations was 6% on April 10. In Payne County an increase in population and parasitism by B. anurus from 1973 to 1974 is evident by comparison of data in Tables VII and X. Evidence of dissemination of B. anurus to areas outside of the release area was obtained in 1974. On April 13, two cocoons of B. anurus were recovered from samples collected in an alfalfa field across the road from the release area.

Close synchrony was not illustrated between peaks of B. anurus and H. postica populations in Payne County (Figure 8). A period of very cold weather with temperatures descending to 20°F for five consecutive nights occurred during the week of March 23, 1974. The cold weather reduced weevil populations across the state and, in the absence of this cold period, H. postica populations would probably have remained at high levels until late March. B. anurus activity may have commenced at an earlier date, narrowing the time lag between population peaks of B. anurus and H. postica.

The data presented concerning B. anurus are encouraging when considering this parasitoid's potential as a successful control agent of the alfalfa weevil in Oklahoma. Its life cycle is fairly well synchronized with that of the alfalfa weevil in Oklahoma. There is no apparent defense mechanism employed by the alfalfa weevil to reduce parasitism by B. anurus in Oklahoma. Puttler (1967) has demonstrated that B. anurus eggs and larvae are not encapsulated by larvae of the alfalfa weevil. In addition, Day (1969) has shown that B. anurus is much less susceptible to attack by Hymenopterous hyperparasites than is B. curculionis. Lastly, there is no evidence of interspecific competition between B. anurus and B. curculionis in Oklahoma.

Tetrastichus incertus

Over 50,000 weevil larvae were reared to pupation during the two-year study period and no T. incertus were recovered. Information on this parasitoid indicate that it is most active in the northeastern United States during June-October when moderate weevil larva populations are present in alfalfa fields (Miller 1970) (Brunson and Coles 1968). In contrast to conditions in the northeast, the peak weevil larval period in Oklahoma usually occurs in March-April with nearly all suitable hosts developing into adults by the end of May. There are no weevil larvae in Oklahoma alfalfa fields during hot weather months of June-

September.

Streams and Fuester (1967) have linked T. incertus diapause with photoperiod and temperature. They have also shown that the greatest activity and parasitism associated with this parasitoid is during June-August in Pennsylvania where it is well established. It is possible that T. incertus is not adapted to the life cycle of the alfalfa weevil in Oklahoma where there are no host larvae present in the summer. The possible adaptation to earlier seasonal activity by T. incertus in Oklahoma might be limited by factors such as photoperiod linked diapause and temperature extremes.

Microctonus aethiops

Three hundred and forty-six weevil adults from Stillwater and 580 weevil adults from Duncan release areas were dissected in 1974 for detection of M. aethiops with negative results. In this case, negative results from one season of sampling will not be interpreted as a failure to establish the parasitoid. While the failure to collect M. aethiops at Stillwater might be attributed to the small host population present when the parasitoids were released, the major problem inherent in any attempt to retrieve M. aethiops, especially so when the parasitoid is present in small numbers, is that the parasitized host adult is mobile. Weevil adults disperse prior to aestivation, and those adults parasitized by M. aethiops in one location may become dispersed over a wide area. Extremely large samples, covering a wide area, may be necessary for detection of the very small number of parasitoids present after only one year of establishment.

TABLE VII

BATHYPLECTES ANURUS RETRIEVAL FROM RELEASE FIELDS IN OKLAHOMA, 1973

Date	Location	# Weevils	# *B. Anurus*	% Parasitism
3/02	Stephens Co.	42	0	0
3/09	"	311	0	0
3/16	"	659	0	0
3/25	"	327	0	0
3/30	"	1109	9	0.8
4/01	"	245	0	0
4/06	"	619	3	0.5
4/13	"	2523	7	0.3
4/20	"	4650	12	0.2
4/28	"	6257	5	0.1
5/03	"	2002	3	0.1
5/09	"	572	0	0
5/18	"	275	0	0
3/15	Payne Co.	236	0	0
3/24	"	341	0	0
4/04	"	949	0	0
4/12	"	917	1	0.1
4/17	"	1187	0	0
4/24	"	3370	13	0.4
5/02	"	4799	2	0.4
5/03	"	2785	19	0.7
5/10	"	973	0	0
5/17	"	521	4	0.8

TABLE VIII

RETRIEVAL OF B. ANURUS AND B. CURCULIONIS FROM RELEASE FIELD IN STEPHENS COUNTY, OKLAHOMA, 1974

		B. ANURUS		B. CURCULIONIS		
Date	# Weevils	Number	% Par	Number	% Par	Total % Par
2/16	25	0	0	0	0	0
2/23	9	0	0	0	0	0
3/01	412	0	0	0	0	0
3/08	1366	0	0	2	0.01	0.01
3/12	2531	35	1.4	9	0.40	1.8
3/15	3190	10	0.3	1	0.03	0.33
3/18	712	11	1.5	1	0.01	1.51
3/22	243	9	4	0	0	4.0
3/27	1637	19	1	8	0.4	1.4
3/31	303	1	0.3	1	0.3	0.6
4/06	1273	6	0.4	2	0.1	0.5
4/12	1963	32	1.7	14	0.7	2.4
4/16	1160	2	0.2	9	0.7	0.9
4/19	1203	2	0.2	29	2.4	2.6
4/24	1472	0	0	83	6.0	6.0
4/26	491	1	0.2	8	1.6	1.8
5/03	371	0	0	41	13.0	13.0
5/09	319	0	0	62	19.0	19.0

TABLE IX

RETRIEVAL OF *B. ANRUS* AND *B. CURCULIONIS* FROM SUB-RELEASE FIELD IN STEPHENS COUNTY, OKLAHOMA, 1974

		B. ANURUS		*B. CURCULIONIS*		
Date	# Weevils	Number	% Par	Number	% Par	Total % Par
3/01	72	0	0	0	0	0
3/08	893	0	0	0	0	0
3/12	1631	0	0	0	0	0
3/15	1356	0	0	3	0.3	0.3
3/18	1204	1	0.1	3	0.2	0.3
3/22	355	0	0	0	0	0
3/27	1699	0	0	4	0.2	0.2
3/31	1492	0	0	1	0.1	0.1
4/06	1636	0	0	0	0	0
4/12	790	1	0.1	0	0	0.1
4/16	800	1	0.1	4	0.5	0.6
4/19	481	0	0	0	0	0
4/24	1189	0	0	9	0.7	0.7
4/26	906	0	0	36	4.0	4.0
5/03	551	0	0	31	6.0	6.0

TABLE X

RETRIEVAL OF *B. ANURUS* AND *B. CURCULIONIS* FROM RELEASE FIELDS IN PAYNE COUNTY, OKLAHOMA, 1974

		B. ANURUS		*B. CURCULIONIS*		
Date	# Weevils	Number	% Par	Number	% Par	Total % Par
3/02	84	0	0	0	0	0
3/09	2000	0	0	2	0.1	0.1
3/11	684	0	0	0	0	0
3/16	845	0	0	1	0.1	0.1
3/19	151	0	0	0	0	0
3/26	699	0	0	2	0.2	0.2
3/30	627	0	0	5	0.7	0.7
4/06	838	12	1.4	10	1.2	2.6
4/10	556	31	6	16	3	9
4/13	3148	81	2.5	34	1	3.5
4/16	456	15	3.2	9	2	5.2
4/18	381	12	3	4	1	4
4/21	3677	69	2	53	1	3
4/25	1485	53	3.5	61	4	7.5
4/28	1144	16	1.3	119	10.4	11.7
5/02	396	3	0.7	55	13.8	14.5
5/06	98	4	4.1	21	21.4	25.6
5/10	16	1	6.2	3	18.7	24.9

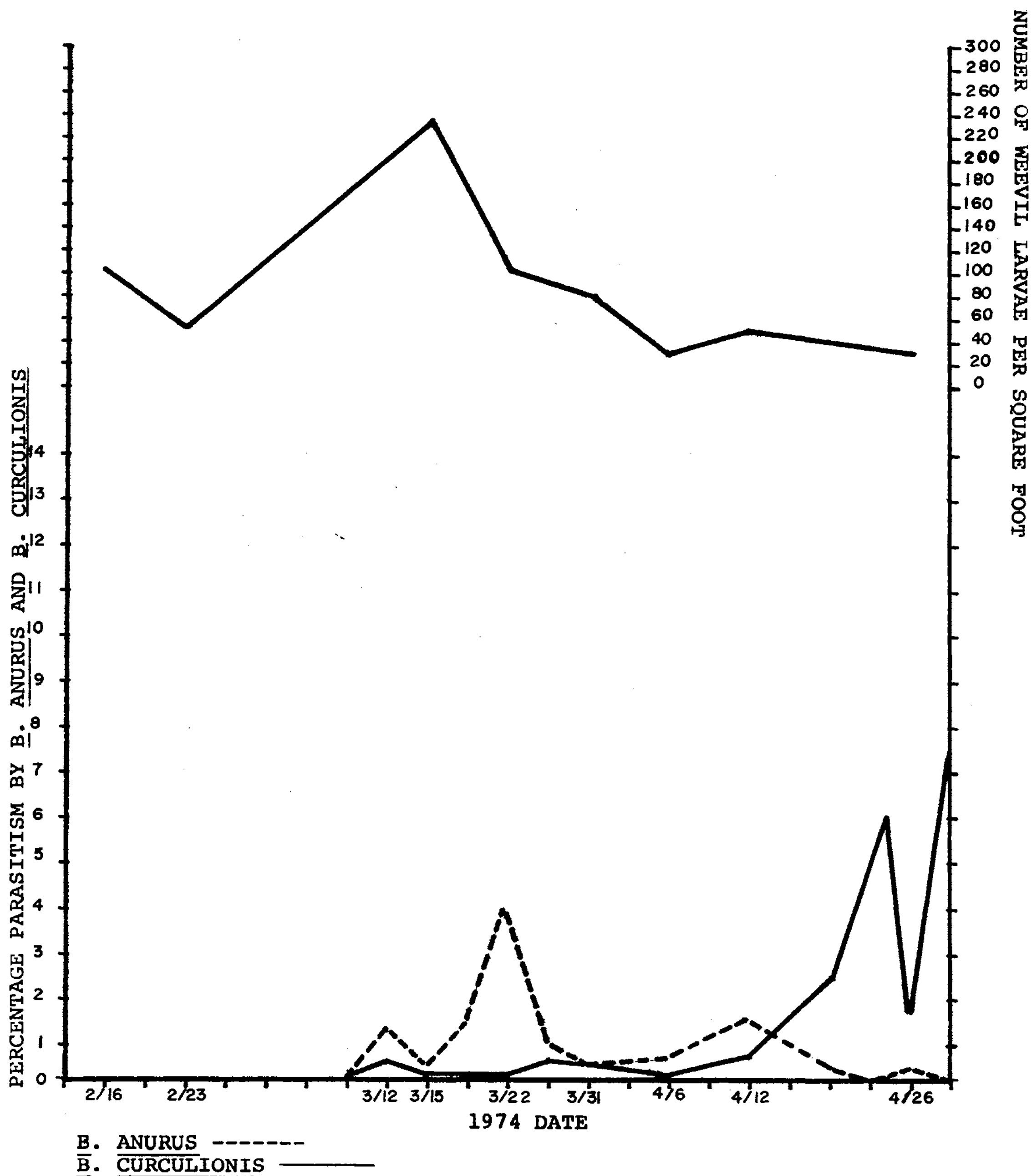

Figure 7. Rate of Parasitism by B. anurus and B. curculionis, and Host Population Level of H. postica in Stephens County Release Field, 1974

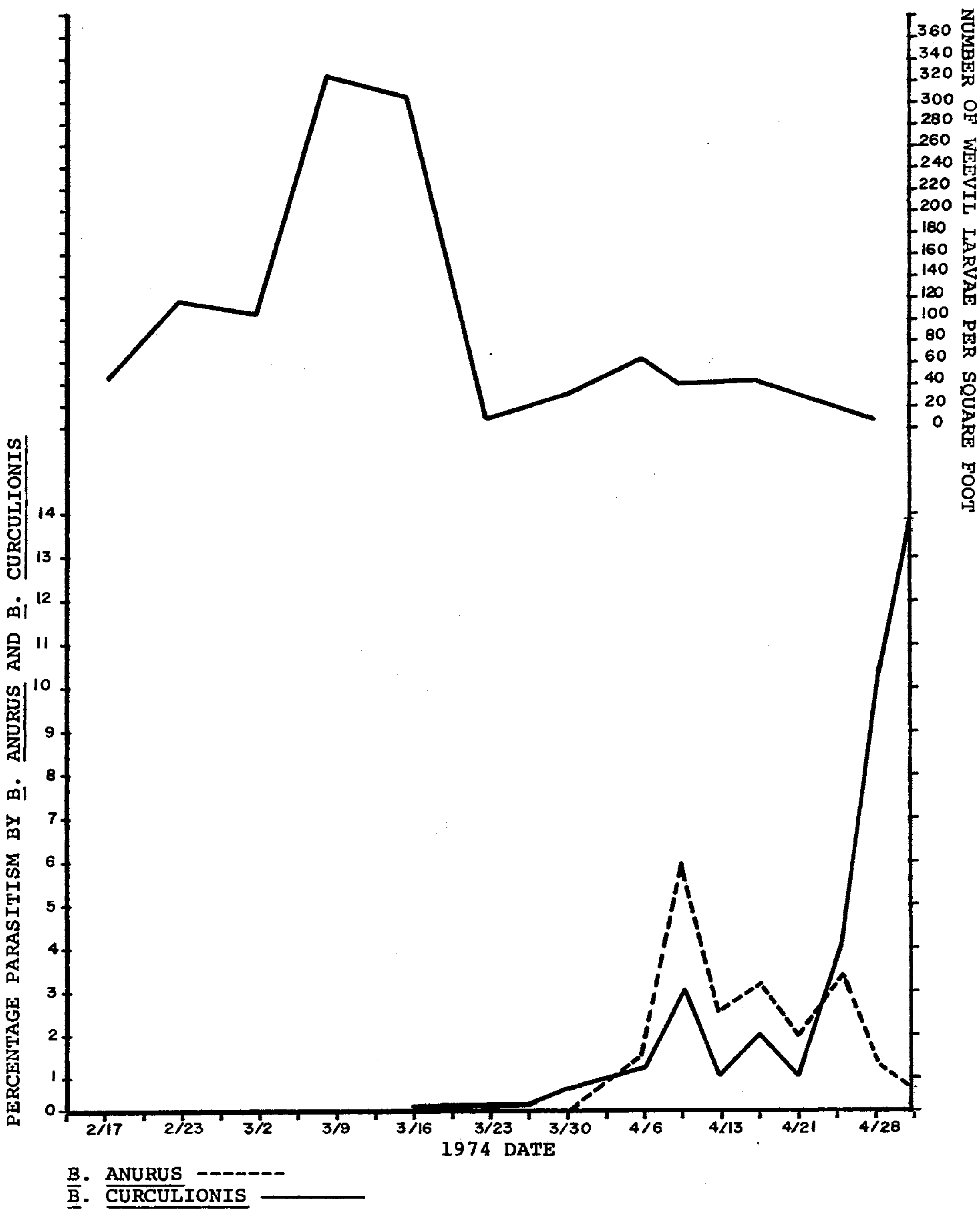

Figure 8. Rate of Parasitism by B. anurus and B. curculionis, and Host Population Level of H. postica in Payne County Release Field, 1974

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