Sampling and survivorship of green cloverworm, Plathypena scabra (Fabricius), pupae in Iowa soybean

Edward John Bechinski
Iowa State University

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SAMPLING AND SURVIVORSHIP OF GREEN CLOVERWORM, PLATHYPENA SCABRA (FABRICIUS), PUPAE IN IOWA SOYBEAN

Iowa State University

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Sampling and survivorship of green cloverworm, *Plathypena scabra* (Fabricius), pupae in Iowa soybean

by

Edward John Bechinski

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major: Entomology

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**PART III: GREEN CLOVERWORM POPULATION DYNAMICS: PUPAL LIFE TABLE STUDIES IN IOWA SOYBEANS**

**Abstract**

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The green cloverworm (GCW), *Plathypena scabra* (F.), is a frequently encountered defoliator of soybeans throughout the eastern half of the U.S. Sporadic GCW outbreaks are the source of much grower concern in the Midwest, and in Iowa, elevate the status of GCW to the major insect pest of soybeans. The development of practical, effective management tactics for this pest will require a detailed understanding of its population dynamics. In particular, if sources of mortality can be identified, then strategies to manipulate and exploit these factors perhaps can be designed.

The life table, a systematic record of age-specific mortality, can provide keys to pest population suppression. Considerable research effort by the Soybean Insects Research Group at Iowa State University has been directed toward the construction of GCW life tables which include larval and adult stages. Although these intensive studies have produced significant advances in understanding GCW population dynamics, they have been accompanied by a corresponding neglect of the remaining life stages. Little is known, for example, about the role of the pupal stage in GCW dynamics. Pupal density and mortality estimates are major data gaps in present life table studies. A sampling program suitable for pupal life table research also is lacking. The research described here was conducted to supply this information. Studies were designed to complement concurrent larval life table research and thereby contribute to a more comprehensive understanding of GCW dynamics. Specific research objectives were as follows:
(1) To determine the microspatial distribution of GCW pupae in soybeans,

(2) To determine sources of variation in pupal dispersion,

(3) To develop a sampling program suitable for pupal life table investigations,

(4) To evaluate alternative pupal sampling techniques,

(5) To identify sources of pupal mortality and to quantify their impact, and

(6) To assess analytically the relative importance and functional role of pupal mortality components.

The results of studies directed toward objectives 1 and 2, 3 and 4, and 5 and 6 are reported here in Parts I, II, and III, respectively.
REVIEW OF LITERATURE

Green Cloverworm

Considerable research effort has been devoted to the biology, ecology, pest status, and suppression of the green cloverworm. Stone and Pedigo (1972b), for example, cited 72 publications concerning these topics. Much of the early literature, however, is limited to qualitative observations or to suggestions for control. Clearly, it is beyond the scope of this review to summarize all but the most pertinent studies. This synopsis particularly emphasizes recent advances in GCW population dynamics, and pupal dispersion, sampling, and survivorship.

In the U.S., GCW larvae are common defoliators of soybeans and other leguminous plants east of ca. 103°W. longitude (Pedigo et al. 1973). On soybean, GCW are categorized as occasional pests. The most serious outbreaks occur in Midwestern soybean production areas every 2 to 5 years, but in the Delta and Atlantic Coast Regions, GCW is considered a minor pest at worst (Pedigo et al. 1981). It seems that GCW life systems (Clark et al. 1967) differ considerably between Midwestern and Southern soybean production areas.

Much of what is known about GCW life systems in the Midwest is the result of research conducted in Iowa since 1968. These studies have been summarized aptly by Pedigo\(^1\) in his hypothesis of GCW dynamics. The hypothesis recognizes that GCW populations can be categorized as either endemic or outbreak. The former expand several-fold from

generation 1 to generation 2, but overall densities remain low. Outbreak populations are much larger during generation 1, frequently exceeding economic-injury levels during soybean growth stages R1 to R3 (flower bloom to pod development). Outbreaks are terminated by epizootics of the entomogenous fungus, *Nomuraea rileyi* (Farlow) Sampson, which reduce survivorship of large larvae during generation 1 and of small larvae during generation 2. Therefore, outbreak and endemic GCW populations are characterized by Indices of Population Trend (*I*) of *I* < 1 and *I* > 1, respectively. The hypothesis further states that GCW do not overwinter in Iowa but immigrate from areas south. The size of the immigrating moth flight determines the larval population configuration.

Not surprisingly, little quantitative information is available about GCW pupae, owing to their unimportance as a direct economic pest and to sampling difficulties. Stone and Pedigo (1972a) reported that the duration of the pupal stage was 9.1 to 10 days, and Hill (1925) and Smith and Franklin (1961) reported 12.1 and 11 days, respectively. Hammond et al. (1979) estimated the threshold of pupal development as 12.2°C (54°F) and determined that 245 T_u are required for pupal development. However, these T_u requirements were calculated by using the egg-to-adult minimum cardinal temperature of 11.1°C (52°F). When calculated on a 12.2°C basis, pupal T_u requirements are 218 rather than 245.

References to previous attempts at sampling GCW pupae could not be located. A few observations have been reported regarding pupal occurrence in agronomic crops. Hill (1925) reported that GCW pupae occur within flimsy cocoons among the surface litter and soil layers in alfalfa. Pedigo et al. (1973) reported a similar situation in soybeans, but also
observed that 10.4% of the pupae occurred within the plant canopy. Biases for canopy height strata were not observed. These data were based on studies with experimental GCW cohorts, and thus, it is not known if the same trends occur among natural populations in open field situations.

Few data similarly have been presented regarding GCW pupal mortality factors. Survivorship studies with experimental GCW cohorts indicated that overall pupal mortality is low (Pedigo et al. 1972b). Pupal mortality, calculated as the difference between late larval and adult densities, ranged from 0 to 15% in screenhouse plots and from 50 to 69% in open field areas. It was believed, however, that lower moth densities recorded in open field plots actually reflected late larval efflux rather than pupal mortality. No attempts were made to directly sample pupae or identify possible pupal mortality agents. Lentz and Pedigo (1974) reported that the tachinid, Winthemia sinuata Reinhard, attacks 5th and 6th stage larvae but sometimes does not cause mortality until the pupal stage. However, parasitization was expressed only in terms of susceptible larvae. Mortality was not allocated to the pupal stage.

Sampling

Accurate insect density data are the foundation of basic ecological research and pest management decision-making. Acquisition of density information, however, remains a problem in entomological field research. Because a complete census is impractical for most insects, sampling programs usually are employed to estimate population levels. Programs
consist of techniques for obtaining counts and procedural plans for employing techniques. Although exact details necessarily depend on the insect population and research objectives, the theoretical and practical discussions on insect sampling presented by Morris (1955, 1960), Strickland (1961), and Southwood (1978) are applicable to any sampling program.

Sampling techniques estimate densities either directly by counting insects or indirectly by recording indices of insect presence. Direct density measurements include absolute estimates, which express density on a unit area basis, and relative estimates, which express density relative to similar counts in space or time but measured in unknown area units. Absolute estimates can be obtained by nearest neighbor techniques, mark and recapture procedures, sampling a known unit of habitat, and by removal sampling. Techniques for relative density estimates include catch per unit effort and trapping procedures. An excellent discussion of these topics is available in Southwood (1978). Kogan and Herzog (1980) should be consulted for specifics regarding sampling methods in soybean entomology.

Sampling techniques can be employed in either intensive or extensive programs. The former are characterized by frequent sampling within relatively small areas, and include life table studies and similar analyses of population dynamics. The latter are characterized by limited sampling over broad areas, and include insect pest surveys and pest management scouting (Southwood 1978).

Procedural plans for employing sampling techniques include 5 basic components (Southwood 1978): (1) choice of universe, (2) sample-unit selection, (3) sample size, (4) sampling pattern, and (5) timing of
sampling. Given basic data on insect biology, spatial arrangement, and sampling variance and cost, these 5 components can be drawn up into a program that provides the desired precision for the minimum sampling effort.

For practical purposes, the sampling universe is defined as the insect's habitat (Morris 1955). Although selection of the appropriate universe usually is obvious, several factors should be considered. First, the universe must be delimited and described clearly to avoid erroneous extension of research conclusions to broader areas. Second, insect dispersion may be biased toward certain habitat subdivisions, such as biases noted among GCW eggs for trifoliate positions on soybean plants (Buntin and Pedigo 1981). Unless such habitats are subdivided properly and sampled in a manner that reproduces observed density gradients, population estimates will contain systematic errors (Southwood 1978).

Morris (1955) proposed 6 criteria for selecting the proper size and shape of sampling units: (1) all units must have equal chance of selection, (2) units are stable (i.e., the no. available to the population does not change), (3) units are used by a constant proportion of the population, (4) units provide a reasonable compromise between costs and precision, (5) units express density in absolute terms, and (6) units can be delineated and collected easily in the field. Formal statistical procedures have been presented by Cochran (1977) for calculating optimal sample-unit sizes, but Southwood (1978) pragmatically observed that because densities fluctuate, a fixed sample-unit size may not be appropriate for all conditions.
Young et al. (1976) appropriately commented that "how many samples are enough... is a constantly recurring question in insect sampling."

Ideally, the number of samples (sample size) should represent a balance between precision required and sampling resources available (Cochran 1977). This compromise is defined by the optimum sample size, the smallest number of samples needed to estimate density with desired precision (Karandinos 1976).

Precision usually is expressed by the standard error as a fraction of the mean, but also can be given by confidence intervals set equal to either a proportion of the mean or some arbitrarily fixed number. The SE/X relationship has been termed relative variation (Pedigo et al. 1972a) and the coefficient of variability (Karandinos 1976). Density estimates with standard errors less than 25% of the mean are satisfactory for extensive sampling programs, but 10% precision is required for intensive studies (Southwood 1978).

The optimum sample size can be calculated from any formula that defines a relationship between precision and population variance. For the 3 precision expressions just mentioned, Karandinos (1976) derived the following formulas:

\[
\begin{align*}
(1) \quad n &= \left( \frac{s}{\bar{x}C} \right)^2 \\
(2) \quad n &= \left( \frac{tS}{DX} \right)^2 \\
(3) \quad n &= \left( \frac{tS}{h} \right)^2
\end{align*}
\]

where \( n \) = optimum no. samples, \( C \) = coefficient of variability, \( t \) = Student's t statistic (usually at 0.05 level), \( D \) = predetermined half-
width of confidence limits (usually 0.1), h = positive fixed number (e.g., $X + 5$ insects), and $S^2$ and $X$ = population variance and mean, respectively. Sampling programs based on these formulas commonly are termed fixed-size plans because sample sizes are entirely fixed, or predetermined, before sampling.

In actual practice, fixed-size plans have limited value because sample-size is given as a function of mean density. Therefore, mean density must be known before sampling to calculate $n$. Although preliminary samples could be taken to assess density, such double sampling defeats the purpose of optimum sample size. An alternative is sequential sampling, in which sample size is variable rather than a predetermined number. Because a decision is made after each sample either to continue or to terminate sampling, sequential plans are much more efficient than fixed-size plans.

Sequential sampling procedures can be categorized as either decision plans or count plans (Allen et al. 1972). Decision plans classify populations into arbitrary density categories (e.g., subeconomic, moderate, or economically-damaging levels). These plans are particularly useful for pest management decision-making, such as deciding if treatment of a pest population is justified, but have little value for intensive studies. Decision plans have been calculated for numerous economically important insects, and in soybeans include velvet bean caterpillar, *Anticarsia gemmatalis* (Hubner), soybean looper, *Pseudoplusia includens* (Walker), GCW, bean leaf beetle, *Cerotoma trifurcata* (Forster), a stink bug complex, and the predatory hemipterans, *Nabis* spp. and *Geocoris* spp. (Kogan and Herzog 1980). Waters (1955) provides a thorough theoretical
and practical discussion of entomological sequential decision plans.

Sequential count plans estimate population levels with predetermined precision, and are well-suited for intensive studies of population dynamics. Although formulas for count plans can be derived easily by substituting the relationship $\bar{X} = \frac{T_n}{n}$ (i.e., mean density = cumulative no. individuals collected/no samples taken) into fixed-size formulas, Kuno (1969) was the first to present such formulas. Because population density, and hence, variance, fluctuate in nature, Kuno re-expressed variance as a function of density, $S^2 = (\alpha + 1)\bar{X} + (\beta - 1)\bar{X}^2$. This functional relationship was derived from Iwao's (1968) regression, $\dot{M} = \alpha + \beta M$, which gives the linear relationship between mean density ($M = \bar{X}$) and Lloyd's (1967) mean crowding parameter, $\dot{M} (M = M + ((S^2/M) - 1))$. Substitution of these expressions into fixed-size Equation 1 produces Kuno's sequential count plan formula

$$T_n = (\alpha + 1)/(D^2 - ((\beta - 1)/n))$$

where $D$ = sampling precision expressed by $SE/\bar{X}$. Because count plans are a relatively recent development, they have received only limited attention. Among soybean insects, count plans have been developed for bean leaf beetles (Boiteau et al. 1979), GCW eggs (Buntin and Pedigo 1981), and the predators Orius insidiosus (Say) and Nabis spp. (Beckinski and Pedigo 1981).

The fourth component of a survey program, sampling pattern, should incorporate random selection of sampling sites to insure unbiased density estimates and to permit statistical analysis of count data. Two approaches are available: simple, unrestricted designs and stratified random de-
signs. Sample sites are selected randomly from the entire universe in simple random plans and from universe subdivisions, or strata, in stratified random plans. Stratified random plans are used widely in ecological studies to minimize sampling error by eliminating strata in which few individuals occur and by insuring a thorough coverage of the universe (Southwood 1978). However, stratified designs often are used uncritically. Unless sound biological data are available to show how strata should be arranged, simple random plans can be expected to be as efficient as arbitrarily designed stratified sampling patterns. Pedigo (1980), for example, reported that completely random designs are acceptable for sampling GCW larvae, although many researchers continue to use stratified plans. The need for stratification can be determined by calculating nested analyses of variance and examining the contribution of each stratum to total variability. Bancroft and Brindley (1958) provide a detailed example of such analyses for the European corn borer, *Ostrinia nubilalis* (Hübner). Cochran (1977) should be consulted for a more complete discussion of these topics.

Selection of an appropriate sampling period is the final component of sampling program design. Obviously, timing depends on the life history and habits of the insect, but sampling objectives also determine when samples should be taken. Life table research and similar intensive studies require regular sampling through the season, but more limited sampling is satisfactory for pest surveillance work and other extensive studies (Southwood 1978). Pedigo (1980), for example, suggested that detailed studies of larval GCW dynamics require sampling on a 2-3 day basis over the entire season. When the objective simply is to detect
impending GCW outbreaks, however, weekly samples taken from July to mid August are adequate.

Life Tables

Life tables—first used in the 18th century—long have been used in the study of human mortality and in applications to nonhuman populations. In the early 20th century, the Gompertz (1947) mortality schedule was introduced as a basis for constructing life tables in ecologically similar species. When constructing life tables for taxa with overlapping generations, life tables can be either precise or imprecise. The former are based on the fate of an imaginary cohort from populations with distinct overlapping generations. The latter are based on the fate of an imaginary cohort from populations with overlapping generations and require determination of age structure (Southwood 1978). Because suitable age-grouping methods often are not available, few life tables have been constructed for insects with widely overlapping generations. Bark beetle (Scolytus sp.) life tables prepared by Beaver (1966) represent an attempt for such insects.

In general, entomological life tables are organized into the
impending GCW outbreaks, however, weekly samples taken from July to mid August are adequate.

Life Tables

Life tables, or age-specific mortality schedules, long have been used in the life insurance industry, but their application to nonhuman populations is a relatively recent development. Deevey's (1947) mortality schedules for Dall mountain sheep represent the first use of life tables in ecological studies. Morris and Miller (1954) constructed the first life table for an insect population. Since these initial studies, life tables, or budgets, have been prepared for numerous insects, and Ives (1964) and Harcourt (1969) have reviewed some of these studies. This interest in the life table approach reflects the critical need for age-specific mortality data to determine causes of population regulation and to design effective management strategies.

Life tables can be either age-specific or time-specific. The former are based on the fate of a real cohort and are restricted to populations with discrete, nonoverlapping generations. The latter are based on the fate of an imaginary cohort from populations with overlapping generations and require determination of age structure (Southwood 1978). Because suitable age-grouping methods often are not available, few life tables have been constructed for insects with widely overlapping generations. Bark beetle (Scolytus sp.) life tables prepared by Beaver (1966) represent an attempt for such insects.

In general, entomological life tables are organized into the
following columns (Harcourt 1969): $x$, the age interval; $l_x$, the number alive at the beginning of the interval; $d_x$, the number dying during the interval; $d_F$, the mortality factor responsible for $d_x$; $100q_x$, percent mortality; and $s_x$, survival rate. Age expectancies ($e_x$ values) typically emphasized in human life tables usually are of little interest to entomologists. Rather, age-specific mortality rates and identification of mortality agents are of primary importance.

The construction of insect life tables has been outlined by Varley et al. (1973) and Southwood (1978). Numbers of individuals passing through a stage ($l_x$ values) are determined by taking a sequence of samples over time or by using accumulative trapping procedures. The latter are limited to insects which can be trapped as they pass from 1 habitat to another at a specific time in the life cycle. Pupal densities of arboreal lepidopterans, for example, often are estimated by trapping mature larvae as they drop from trees to soil litter pupation sites (Ives 1964). Counts of individuals entering traps are accumulated and the sum is entered directly in life tables.

When trapping procedures are not appropriate, $l_x$ values are determined from a series of density estimates taken on successive days. If all individuals are in the same developmental stage at a single point in time, then a single census at this point will be sufficient to estimate density. Indirect sampling procedures have been used in a similar manner by delaying sampling until all individuals have passed through a stage. Morris and Miller (1954), for example, estimated densities of adult spruce budworms, *Choristoneura fumiferana* (Clemens), by sampling pupal cases after moths had emerged.
In most instances, developmental stages overlap and it is necessary to integrate density data from many sampling periods into a single $l_x$ value. At least 8 integration procedures have been proposed, and they differ greatly in their underlying assumptions, data requirements, and mathematical complexity. Southwood (1978) contains a complete review of these procedures.

The simplest, most robust, and probably the most widely used integration procedure is graphical summation (Southwood 1978; Helgesen and Haynes 1972). This method involves plotting population curves and determining the area under the curves. The area represents the total incidence, or total number of times individuals from an age class are observed. By dividing total incidence by the average time an individual remains in a stage, an estimate is obtained of population density at the median age of the stage. Mean developmental time usually is used as an estimate of average time, but because an individual's effective life also depends on the distribution and intensity of mortality, these survivorship factors can influence the accuracy of graphical summation. If mortality is severe at the beginning of a stage, the average individual actually lives less than the mean developmental time and densities will be underestimated. Similarly, if mortality occurs entirely at the end of the stage, then graphical summation provides a reasonable estimate of numbers passing through the stage. Although graphical summation actually measures densities at the median age, estimates usually are used as numbers entering. Ruesink (1975), however, suggested that an alternative is to establish overlapping age classes in life table $x$ columns, such as from midfirst to midsecond stage larvae, rather than
within the first and second larval stages.

Given a series of $l_x$ estimates for each life stage, age specific mortality (life table $d_x$ terms) can be calculated as the difference between successive $l_x$ values. Because these differences may reflect dispersal and sampling error in addition to mortality, mortality agents must be identified and their impact quantified to substantiate $d_x$ terms. In general, parasitization and disease incidence are measured directly from host collections, rearings, and dissections. Predation is more difficult to assess directly, but can be calculated as the residual mortality remaining after accounting for factors that were observed directly. Varley and Gradwell (1968), for example, calculated pupal predation rates for the winter moth, *Operophtera brumata* (L.), as the unexplained pupal mortality left after subtracting pupal deaths due to parasitism. Varley et al. (1973) particularly emphasize the importance of calculating such residuals, stating that "many workers have found... that the residuals are more important than many of the mortality factors which are easy to measure directly."

Predation has been studied directly by using observational and experimental approaches. The appearance of dead insects, particularly the egg and pupal stages, has been used to estimate not only predation but also parasitism and other factors. Experimental methods have included exclusion techniques which restrict access of certain predators to prey. Buckner (1959), for instance, utilized artificial cohorts and mammal exclusion cages to quantify shrew predation of sawfly cocoons. East (1973) combined similar exclusion techniques with pitfall sampling to identify predators of winter moth pupae.
Like predation, the impact of climatic factors has been examined both observationally and experimentally. In life table studies of the cereal leaf beetle, *Oulema melanopus* (L.), differences between density estimates taken before and after thunderstorms were used to determine larval mortality caused by rainfall (Shade et al. 1970). Latheef et al. (1979) conducted laboratory experiments in environmental chambers to quantify desiccation among pupal alfalfa weevils, *Hypera postica* (Gyllenhal).

From the foregoing discussion, it may be concluded that life table studies are major research endeavors and should receive careful consideration before being undertaken. Particular attention must be given to the design of sampling programs which accurately estimate both density and mortality for all life stages. It cannot be overemphasized that development of an appropriate program is neither simple nor inexpensive. In addition, a series of life tables, covering a range of density levels and environmental conditions, are required to understand population dynamics. Therefore, the researcher must be committed to long-term studies. Although none of these problems are insurmountable, they must be resolved before beginning life table studies.

Compilation of life tables is not an end in itself, but instead appropriate analyses are required to fully understand and utilize life table data. As Morris (1963) observed, such analyses "reveal how much (or how little) we understand about the population dynamics of a species... and show the probable effects on population trend of manipulating variables that are controllable by man."

At least 3 basic techniques have been used to analyze life table
data: key factor analysis, determination of density relationships, and multivariate procedures (Varley and Gradwell 1970). Although multivariate techniques have been used widely to analyze population data and to develop models that predict population changes (e.g., Morris 1963), Varley and Gradwell (1970) have criticized this approach, stating that "these [multivariate] models, at their very best, result in crude descriptions but explain nothing." Because these analyses do not provide insights into the biological mechanisms of population change, they cannot suggest ways in which mortality factors can be manipulated in management strategies. Therefore, Varley and Gradwell suggest that key factor methods and detection of density relationships are preferred to multivariate analyses.

Morris (1959) introduced the concept of key factor analysis. This concept recognizes that although many factors contribute to total mortality, usually only a few agents, termed key factors, primarily account for changes in population size. Morris proposed analytical techniques for incorporating key factors into predictive population equations, and although his methods have been criticized on both biological and statistical grounds (e.g., Varley and Gradwell 1970; Luck 1971; Kuno 1973), they did stimulate further advances in key factor analysis. Primary among these are the methods of Varley and Gradwell (1968).

Varley and Gradwell's key factor analysis is based on the relationship

\[ \text{Generational survival} = S_G = S_1 \times S_2 \times \ldots \times S_n \]

where \( S_n \) values are age-specific \( S_x \) terms from life tables. To reduce
variance and to improve linearity prior to analysis, Equation 5 is re-
expressed in terms of k-values, or logarithmic measures of the killing
power of a mortality factor:

\[
(6) \text{Total mortality} = K = k_1 + k_2 + \ldots + k_n.
\]

Individual k-values are calculated as the difference between successive
\[\log_1 x\] values, and are equivalent to \[\log S_x\].

The key factor most associated with total mortality can be identified
in several ways, but the simplest is to plot changes in k-values with
respect to total K for as many generations as possible, and then to
visually determine which factor contributes most to variation in total
mortality. Alternatively, correlation analyses and regression procedures
(Podoler and Rogers 1975) can be used to examine relationships between
individual k-values and K. Strictly speaking, however, these statistical
tests are invalid because k-values and K usually are intercorrelated
(i.e., any errors made in estimating submortalities also are incorporated
into total mortality estimates). However, as long as it is realized
that correlation and regression procedures do not test the actual im-
portance of each submortality, but rather test their contributions to
changes in K, these methods can be employed to identify key factors
(Podoler and Rogers 1975).

Key factor analysis can be applied only to mortality factors which
act in succession. When agents act simultaneously, their impact can
be expressed by m-values (mortality values) (Beaver 1965). The m-values
are calculated as are k-values, but differ in being nonadditive (i.e.,
In life table analyses, the detection of density relationships also is critical to understanding the biological mechanisms of population change. Life table mortality components fall into 1 of 4 categories (Varley and Gradwell 1970): direct density-dependent mortality, inverse density-dependent mortality, delayed density-dependent mortality, and density-independent mortality. The first 2 kill an increasing and decreasing proportion of the host population as host density increases. Inverse relationships are rare, but do occur with nonspecific, non-synchronized parasitoids. Varley and Gradwell (1970) feel that direct density-dependent mortalities regulate populations (i.e., stabilize population changes) and that inverse factors influence populations by contributing to instability. Delayed density-dependent mortality is based on the theory that a parasitoid's rate of increase, not host mortality, is proportional to host density (Varley 1947). Cyclical changes are expected between host and parasitoid, with the latter theoretically lagging the host by 1 quarter of a cycle (i.e., maximum host mortality occurs 1/4 of a cycle after peak host density). Only specific, synchronized parasitoids have been observed to act in a delayed manner. Density independent mortality, as implied, does not respond to host density but instead produces a variable amount of mortality. Varley and Gradwell (1970) believe that density-independent factors often are the key factors associated with population change.

Varley and Gradwell (1968, 1970) extended the use of k-values to the detection of functional relationships. Conceptually, linear regression of individual k-values on log $1 - x$ values is a straightforward test.
for density dependence. This test simply is the logarithmic equivalent of regressing % mortality against population density. Direct and inverse density-dependent mortality are identified by $\beta > 0$ and $\beta < 0$, respectively, while $\beta = 0$ designates density-independent mortality. Delayed density-dependent factors similarly result in $\beta = 0$, but can be distinguished by the anticlockwise spiral created when data points are linked serially in time sequence.

Although these regression procedures may suggest how mortality factors function, in the strictest sense, they are invalid because $k$-values and $\log l^x$ terms are not independent (i.e., $k = \log l^x_{t+1} - \log l^x_t$) (Varley and Gradwell 1970). Statistically significant regressions might reflect underlying biological mechanisms or simply could be artifacts of sampling error. If density dependence is suggested, regressions of log density after mortality against log density before mortality (i.e., $l^x_{t+1}$ on $l^x_t$) should be calculated. Direct and inverse density-dependence is shown by $\beta < 1$ and $\beta > 1$, respectively, and density-independence is indicated by $\beta = 1$. However, even this test may be statistically invalid because linear regression assumes that the independent variable (i.e., $\log l^x_t$) is measured without error. Although $l^x$ terms necessarily include sampling errors, Kuno (1973) suggested that if error (SE/$\bar{x}$) is less than 10%, then these procedures do not violate statistical assumptions. An alternative is to calculate the regression $l^x_t$ on $l^x_{t+1}$ and $l^x_{t+1}$ on $l^x_t$. If both lie on the same side of $\beta = 1$ and also differ significantly from $\beta = 1$, then a formal proof of density-dependence has been made (Varley et al. 1973). Sokal and Rohlf (1969) discuss other solutions. Southwood (1978) aptly has
summarized the problems of detecting density relationships: "one must conclude that the demonstration of density dependence from census data is fraught with difficulties; in particular, failure to detect it in no way proves its absence."
PART I: MICROSPATIAL DISTRIBUTION OF PUPAL GREEN CLOVERWORMS IN IOWA SOYBEAN FIELDS
ABSTRACT

The microspatial distribution of pupal green cloverworms, *Plathypena scabra* (F.), was studied intensively in six soybean fields during 1979 and 1980. Pupae were recovered from the plant canopy and soil-litter surface but never from subsurface soil layers. Litter-layer sites accounted for 90% of the pupae collected. Pupal densities were biased with respect to directional aspect, with more pupae located in southern or eastern aspects than northern or western sites. Pupal densities also varied inversely with distance from the soybean row. Both the quantity of litter cover and population density influenced pupal distribution. Implications for pupal sampling are discussed.
INTRODUCTION

The green cloverworm (GCW), *Plathypena scabra* (F.), is a widely distributed defoliator of U.S. soybeans. Because development of effective management strategies for this pest will require a thorough knowledge of population dynamics, GCW life tables are being constructed in Iowa. Initially, studies were restricted to the larval and adult stages because sampling procedures for the remaining stages were not available. However, investigation of GCW egg spatial patterns and the development of an egg sampling program (Buntin and Pedigo 1981) has left information about the pupal stage as a primary data gap in life table studies.

Relatively little is known about GCW pupal occurrence and distribution in soybeans. Hill (1925) reported that in alfalfa fields, GCW pupae occur among the ground litter within loosely constructed cocoons of silk and soil debris. Pedigo et al. (1973) reported a similar situation in soybeans but also observed pupae within the soybean plant canopy.

The objectives of research reported here were to determine the microspatial distribution of GCW pupae in soybeans and to determine sources of variation in dispersion patterns. These data are essential to the development of pupal sampling plans, and, therefore, are critical to the completion of the GCW life table effort.
METHODS AND MATERIALS

GCW pupae were sampled near Ames in 2 soybean fields during 1979 and in 4 fields during 1980 (varieties breeder soybeans, 'Corsoy', 'Cumberland', 'Harcor', 'Pella', and 'Hodson 78', respectively). Rows were planted on 76-cm centers, and total field size ranged from 20 to 32 ha. Sampling design was stratified random. Four 0.2-ha blocks (10 rows x 262 m) were located in each field, with Block 1 adjacent to the field margin and the remaining blocks parallel at successive 30.5-m intervals. Blocks were divided into 4 equal-sized plots.

Samples were taken at 4 random sites/plot at fields 1 and 2 during 1979 and 1980, and at 2 random sites/plot at fields 3 and 4 during 1980, for a total of 64 and 32 samples/date, respectively. Manpower limitations during 1980 reduced sample size at fields 1 and 2 to 2 random samples/plot after Aug. 27. Sampling began July 25, 1979, and July 10, 1980, and continued on a weekly basis for the duration of pupation. Total sampling periods were 7, 6, 12, 13, 12, and 8 weekly dates at field 1, 1979; field 2, 1979; field 1, 1980; field 2, 1980; field 3, 1980; and field 4, 1980, respectively.

The sampling unit was a 60 x 60-cm area centered over the soybean row. This size was selected so that the sampling-unit would extend approximately to the midpoint between adjacent rows. Plants within the unit were removed and examined, and pupal position and height within the canopy were recorded. Wire templates then were placed on the soil surface to divide the sample-unit into 36 contiguous 10 x 10-cm quadrats.
Ground litter and the immediate soil surface were searched, and pupal location was mapped. The possibility that pupae might occur below the immediate soil surface was investigated by taking soil-core samples. Pupation below the soil surface layer never has been reported in the literature, and practical field experience further suggested that GCW larvae cannot burrow into the soil. Therefore, core sampling was limited to a single date (July 26, 1979) at field 2. The individual 10 x 10-cm quadrats at 1 random sampling site/plot were removed systematically in 5-cm layers to a total depth of 15 cm. These 1728 soil cores were screened and examined for GCW pupae.

Soil-litter cover was quantified by using a modified point-quadrat method (Greig-Smith 1964). A Plexiglas® sheet, containing 25 systematically arranged holes / 10 x 10-cm quadrat, was placed over each quadrat before making pupal inspections (Fig. 1). Litter cover was sighted through each hole and percent litter cover was calculated. Point-quadrat samples were taken at 1 random sampling-site in each plot on all sampling periods during 1979, but were limited to the initial 5 dates at fields 1 and 2 during 1980.

\[1\text{Dr. L. P. Pedigo, Dept. of Entomology, Iowa State University, Ames, IA., pers. comm.}\]
Fig. 1. Point-quadrat procedure utilized to quantify soil surface litter cover

A wire template has been placed on the surface to divide the 60 x 60-cm sampling unit into 36 contiguous 10 x 10-cm quadrats. Litter cover on each quadrat is determined by sighting surface debris through systematically arranged holes drilled in a Plexiglas sheet.
RESULTS AND DISCUSSION

Pupal Distribution

Examination of plant-canopy, litter-layer, and soil-core sampling units confirmed that GCW pupae were limited to the first 2 sites (Table 1). Pupae never were recovered from soil cores. Litter-layer sites were preferred in all fields during both years. Plant-canopy sites accounted for 10.5% of the pupae collected, which corresponds closely to 10.4% within the canopy reported by Pedigo et al. (1973). Table 1 also shows that pupal densities varied considerably between fields, but that overall densities were much greater in 1979 than 1980.

The distribution of GCW pupae collected from the plant canopy is shown in Table 2. An ANOVA with orthogonal comparisons, computed on the original count data pooled from all fields, indicated that significantly (p < 0.05) more pupae occurred on leaves than on the remaining plant sites. Approximately 60% were found in flimsy, silken cocoons webbed to leaves. On an individual field basis, however, this preference was significant only at fields 1 and 2 during 1979. Densities were low and variable at the other fields, and statistically significant differences among pupal locations on plants were undetectable. More than 80% occurred in the middle and lower canopy strata. Pedigo et al. (1973), in contrast, found "equal numbers" in the 3 canopy strata. During 1979 and 1980, however, pupal density differences among height strata were not significant.
Table 1. GCW pupal sampling program summary, Ames, IA, 1979-1980

<table>
<thead>
<tr>
<th>Site</th>
<th>No. units examined&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total no. pupae collected</th>
<th>% of pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant-canopy, litter-layer&lt;sup&gt;b&lt;/sup&gt;, soil cores</td>
<td></td>
<td>Plant canopy</td>
</tr>
<tr>
<td>1979</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>448</td>
<td>436</td>
<td>6.7</td>
</tr>
<tr>
<td>Field 2</td>
<td>384</td>
<td>182</td>
<td>22.0</td>
</tr>
<tr>
<td>1980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>640</td>
<td>48</td>
<td>8.3</td>
</tr>
<tr>
<td>Field 2</td>
<td>640</td>
<td>137</td>
<td>8.8</td>
</tr>
<tr>
<td>Field 3</td>
<td>384</td>
<td>86</td>
<td>9.3</td>
</tr>
<tr>
<td>Field 4</td>
<td>256</td>
<td>6</td>
<td>16.7</td>
</tr>
<tr>
<td>Totals</td>
<td>2752</td>
<td>1728</td>
<td>10.5%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sample-unit size = 60-cm row, 60 x 60-cm litter area, and 10 x 10 x 5-cm core, for plant canopy, litter layer, and soil pupation sites, respectively.

<sup>b</sup>n plant-canopy units and n litter-layer units (e.g., 448 canopy and 448 litter units were examined at field 1, 1979).
Table 2. Distribution (%) of GCW pupae collected on soybean plants, Ames, IA, 1979-1980

<table>
<thead>
<tr>
<th>Site</th>
<th>No. pupae collected</th>
<th>Location on plant (%)</th>
<th>Position in canopy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stems&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Leaves</td>
</tr>
<tr>
<td>1979</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>29</td>
<td>27.6</td>
<td>72.4</td>
</tr>
<tr>
<td>Field 2</td>
<td>40</td>
<td>22.5</td>
<td>62.5</td>
</tr>
<tr>
<td>1980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>4</td>
<td>25.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Field 2</td>
<td>12</td>
<td>33.3</td>
<td>25.0</td>
</tr>
<tr>
<td>Field 3</td>
<td>8</td>
<td>62.5</td>
<td>25.0</td>
</tr>
<tr>
<td>Field 4</td>
<td>1</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Totals</td>
<td>94</td>
<td>28.7%</td>
<td>58.7%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Stems include main stem, branches, and petioles.

<sup>b</sup>Position in canopy not measured at fields 3 and 4, 1980.
Pupal distribution among ground litter sites was examined with respect to directional aspect and distance from the soybean row (Table 3). Soybean rows were oriented on east-west axes at fields 1 and 2 during 1979 and 1980, and on north-south axes at fields 3 and 4 during 1980. Therefore, pupae could occupy northern or southern exposures at the first 4 fields, and western or eastern exposures at the last 2 sites. An ANOVA computed on the original count data showed that significantly more pupae were located on the south side of the soybean row than on the north side at fields 1 and 2, 1979. The same trend occurred at field 1, 1980, but density differences were not significant. Selection of northern exposures at field 2, 1980, was not statistically significant. No statistical differences could be detected between eastern and western exposures at fields 3 and 4, although data combined from both fields suggest a trend toward greater densities on the east side of the row.

Pupal densities varied inversely with distance from the soybean row (Table 3). More than half of the pupae were recovered within 10 cm of the plants, and more than 80% were within 20 cm. An ANOVA with orthogonal comparisons, based on the original count data, was calculated for each field. Pupal densities within 10 cm of the row were significantly greater than densities beyond 10 cm at all sites except field 4, 1980. Low densities at field 4 precluded detection of differences, although the inverse relationship between density and distance was observed. Densities at 10 to 20 cm did not differ significantly from those at 20 to 30 cm, with the exception of field 1, 1979.
Table 3. Distribution (%) of GCW pupae collected in the soil litter as related to directional aspect and distance from the soybean row, Ames, IA, 1979-1980

<table>
<thead>
<tr>
<th>Site</th>
<th>No. pupae collected</th>
<th>Aspect&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Distance from row (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>South vs north</td>
<td>West vs east</td>
</tr>
<tr>
<td>1979</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>407</td>
<td>56.3</td>
<td>43.7</td>
</tr>
<tr>
<td>Field 2</td>
<td>142</td>
<td>67.6</td>
<td>32.4</td>
</tr>
<tr>
<td>1980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>44</td>
<td>52.3</td>
<td>47.7</td>
</tr>
<tr>
<td>Field 2</td>
<td>125</td>
<td>46.4</td>
<td>53.6</td>
</tr>
<tr>
<td>Field 3</td>
<td>78</td>
<td>-----</td>
<td>44.9</td>
</tr>
<tr>
<td>Field 4</td>
<td>5</td>
<td>-----</td>
<td>60.0</td>
</tr>
<tr>
<td>Totals</td>
<td>718</td>
<td>56.5%</td>
<td>43.5%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Aspect = north or south side of soybean rows oriented east/west, and west or east side of soybean rows oriented north/south.
Sources of Variation

Because GCW pupae seek out protected areas for pupation (Hill 1925), it seemed likely that litter cover could account for variation observed in pupal distribution. Fig. 2 shows that pupae generally were located in quadrats with greater than average litter cover. Deviations were greatest when the average litter cover per site was low and large differences existed in cover values among quadrats. As the season progressed, deviations decreased because all quadrats were equally litter covered and thus equally suitable as pupation sites. Over the entire season, litter cover on quadrats where pupae were collected deviated from average cover + 18.4% (107 observations), + 22.9% (34 observations), - 20.0% (4 observations), and + 2.2% (3 observations) at field 1, 1979; field 2, 1979; field 1, 1980; and field 2, 1980, respectively. T-tests, however, showed that these differences were statistically significant only at field 1, 1979. When data were pooled from all 4 fields, the analysis showed that litter cover on quadrats with pupae was 17.6% greater than mean cover and that this difference was statistically significant.

Given the preference of GCW pupae for highly littered sites, variation in ground cover among sites should explain the observed preferences for directional aspect and distance from the row. Mean ground cover values on southern exposures deviated + 10.4%, - 0.3%, - 1.3%, and - 3.0% compared to northern exposures at fields 1 and 2, 1979, and fields 1 and 2, 1980, respectively. These differences are statistically significant only in the first case. Therefore, it seems that greater litter cover on
Fig. 2. Relationship between litter cover on quadrats where pupae were collected and mean % litter cover per quadrat

Cover on quadrats with GCW pupae is expressed as % deviation from mean % litter cover per quadrat. Each data point is the mean from a weekly sampling period.
Cover on quadrats with pupae (% deviation from $X_{x}$/quadrat/date)

Mean percent litter cover
($X_{x}$ per 100 cm$^2$ quadrat per date)

- □ = field 1, 1979
- • = field 2, 1979
- ▲ = field 1, 1980
- △ = field 2, 1980
southern exposures at field 1, 1979, accounts for the significantly
greater pupal densities observed there. Likewise, the lack of cover
differences between aspects at fields 1 and 2, 1980, also accounts for
the lack of significant pupal density differences between aspects at these
sites. However, factors other than litter cover were responsible for
aspect preferences at field 2, 1979, because both exposures contained
equal cover, but densities were biased toward southern sites.

Variation in pupal density with distance from the row similarly is
explained by litter cover. Like pupal density, litter cover at all 4
fields varied inversely with distance from the soybean row. Variance
analyses with orthogonal comparisons indicated that cover within 10 cm
of the row was significantly greater than cover beyond 10 cm at all
fields, and that cover at 10 to 20 cm was significantly greater than
cover beyond 20 cm at all sites except field 2, 1980. Although litter
cover was not quantified at fields 3 and 4, 1980, it is likely that cover
would have accounted for the distribution patterns observed at these loca-
tions.

Population density also influenced pupal distribution. Pupal occur-
rence on southern and eastern aspects was greatest when densities were
low (<10,000/ha), but these preferences decreased curvilinearly as
density increased (Fig. 3). Approximately equal numbers of pupae occurred
on either side of the soybean row when densities were greater than 40,000/
ha. Because regression models describing selection of southern or eastern
exposures were essentially identical (i.e., $\%_{\text{south}} = 242.48 \times (\text{density})^{-1.16}$
Fig. 3. Relationship between pupal GCW density and selection of directional aspect, Ames, IA, 1979-1980

Each data point is the mean of 32 or 64 weekly samples at each site.
GCW pupal density (x 10000)/ha

% pupae on southern or eastern aspects

$Y = 239.5 X^{-16}$

$R^2 = .45, N=44$
and $\%_{\text{east}} = 230.57 \ (\text{density})^{-0.16}$, a single analysis was calculated by pooling data from all fields. This analysis showed density accounted for 45% of the total variation observed in pupal distribution with respect to aspect.

Similar relationships could not be demonstrated between density and distribution with respect to distance from the row (Fig. 4). However, with the exception of several outlying points located at the origin, the data suggest that density and distance preferences actually were related curvilinearly as were density and aspect selection. Aspect and distance preference responses suggest that "mutual interference" among individuals competing for pupation sites results in the selection of less than preferred sites when densities are high.

Factors accounting for selection of plant-canopy pupation sites were not obvious. It had been expected that as litter cover increased, the proportion of the population in the canopy would decrease because more potential soil litter sites would be created. Significant negative correlations could not be shown, however, between litter-cover and selection of canopy pupation locations. Nor could any relationship be detected between population density and canopy preferences. A plausible explanation is that these pupation preferences reflect innate, genetically predetermined behavior rather than a response to environmental factors.

Implications for Pupal Sampling

These studies illustrate several factors that must be considered in the design of GCW pupal sampling plans. First, the sampling universe
Fig. 4. Relationship between pupal GCW density and distance preferences, Ames, IA, 1979-1980

Each data point is the mean of 32 or 64 weekly samples at each site.
should include both the plant canopy and the soil surface, but need not include subsurface soil layers. Second, because GCW pupae within the soybean canopy did not exhibit stratification by height, it is not necessary to examine the entire plant. A sampling-unit restricted to 1 height stratum would provide unbiased density estimates with considerable savings in manpower sampling costs. Third, Southwood (1978) pointed out that systematic errors arise in density estimates when samples are taken randomly from populations biased toward certain habitat subdivisions. Density estimates of GCW pupae located in the litter layer might contain such systematic errors if samples were taken without regard for directional aspect or distance from the soybean row. These errors can be avoided, however, if the sampling unit reproduces the gradient observed in the habitat. Therefore, the sampling unit should include both sides of the soybean row and also should extend to the midpoint of the adjacent row. Any other sampling unit would ignore potential density gradients and would be subject to systematic bias. Finally, the choice of plant-canopy and litter-layer sampling units is well-founded because both also fulfill the 6 criteria proposed by Morris (1955) for the selection of an appropriate sampling unit.
PART II: DEVELOPMENT OF A SAMPLING PROGRAM FOR PUPAL GREEN CLOVERWORM LIFE TABLE STUDIES IN SOYBEANS AND EVALUATION OF ALTERNATIVE SAMPLING PROCEDURES
ABSTRACT

Intensive surveys of pupal green cloverworms (GCW), *Plathypena scabra* (F.), were conducted in 6 soybean fields during 1979 and 1980 to develop a sampling program suitable for life table research. Nested analyses of variance (NANOVA) demonstrated that differences within soil-litter sample sites and among plant-canopy samples accounted for > 95% of the total variation observed in pupal densities on the soil surface and in the canopy, respectively. NANOVA also indicated that simple random sampling plans are satisfactory for pupal surveys. Calculation of an optimal sampling-unit size showed that a 50 x 60-cm area, centered over the soybean row, represented the best compromise between sampling cost and precision. Sequential count plans, based on Taylor's Power Law, were calculated for estimating pupal densities with 3 predetermined precision levels.

Evaluation of fixed-time and single-date pupal sampling techniques suggested that the former method is a potential time-saving alternative to intensive survey procedures and that the latter method is too imprecise for GCW pupal life table studies.
INTRODUCTION

The green cloverworm (GCW), *Plathypena scabra* (F.), is a serious but sporadic defoliator of Midwestern soybeans. Life table studies are underway in Iowa to better understand and manipulate factors regulating GCW populations. Because these studies require highly accurate density estimates, design of suitable sampling programs received strong emphasis. Sampling plan development involves 5 basic components (Southwood 1978): (1) choice of sampling universe, (2) sample-unit selection, (3) calculation of required sample-size, (4) determination of sampling pattern, and (5) timing of sampling. Given basic data on insect biology, spatial arrangement, and sampling variance and cost, these 5 components can be organized into a program that provides the required precision for the minimum sampling effort.

The lack of basic, prerequisite data has limited GCW life table sampling to the larval and adult stages. Studies of pupal GCW microspatial patterns reported in Part I of this thesis provide sufficient data to select both an appropriate universe and a sampling-unit for a pupal survey program. The research reported here is a continuation of the initial studies. Research objectives were to further define pupal sampling-plan components, to develop a sampling program suitable for pupal life table investigations, and to evaluate alternative pupal sampling techniques.
METHODS AND MATERIALS

Intensive Sampling Procedures

Pupal sampling procedures were described in Part I. The 10 x 10-cm soil-litter quadrats were grouped into 6 contiguous 10 x 60-cm subsamples units to permit calculation of optimal sample-unit size. These subsample units were oriented perpendicularly to the row (i.e., units extended 30 cm on both sides of the row) to avoid systematic errors in pupal density estimates.

Indirect Sampling Techniques

Because the intensive survey procedures require considerable expenditures of time and money, 2 indirect, less labor-intensive techniques were evaluated as possible alternatives: (1) a fixed-time plan, and (2) a single-date plan.

The fixed-time procedure limited examination of the 60 x 60-cm unit to 2 man-minutes. The soybean canopy was sampled first by beating the plants over a polyethylene ground cloth for 5 sec to dislodge pupae. The ground cloth was removed and the soil surface was searched during the remaining time. Two randomly located samples were examined at each plot (32 total samples/date), and samples were taken on a total of 6 dates in fields 1 and 2 during 1979.

The single-date plan utilized the same field design, sampling procedures, and sample size as the intensive survey, but differed in that sampling was limited to single date following completion of moth emergence.
Pupal exuviae were collected and classified into survivorship categories (e.g., moth emerged, parasitoid emerged, etc.) based on pupal appearance. Samples were taken at the end of GCW generations 1 and 2 in all 6 fields during 1979 and 1980. Morris and Miller (1954) used similar delayed-sampling procedures to estimate pupal densities for spruce budworm, *Choristoneura fumiferana* (Clemens), life table studies.
RESULTS AND DISCUSSION

Sampling Plan Design

**Within-field variation in pupal densities**

Sources of variation in pupal densities must be identified to design an efficient sampling program. Therefore, a nested analysis of variance (NANOVA) was calculated for each weekly data set to examine the respective contributions of blocks, plots, 60 x 60-cm sampling sites, and 10 x 60-cm subsample units to total observed variation. Means from these analyses (Table 4) showed that, in general, differences among ground-litter subsample units and among plant-canopy sampling sites accounted for > 95% of the total variation in pupal densities on the soil surface and on plants, respectively. These results suggest that substantial gains in precision could be realized by selection of the proper sample-unit size.

Block variation, which reflected differences in pupal densities at successive 30.5-m intervals from the field margin, contributed little to the total. Because differences among blocks seldom were significant, "border effects" can be ignored in GCW pupal surveys. Plots similarly contributed little to total variation, but differences in pupal densities among plots were statistically significant in 22% of the ground-litter data sets. Because field-survey staff were assigned the same plot number within a sampling period, differences among plots primarily reflected differences in skill levels among samplers rather than aggregation due to biological processes. These sampler effects seldom were detected in plant-canopy pupal density estimates.
Table 4. Results (means) of nested analyses of variance (NANOVA) computed for each sampling date on pupal GCW samples from 6 soybean fields, Ames, IA, 1979-1980

<table>
<thead>
<tr>
<th>Variance component</th>
<th>% of total variation contributed by component, and (% of data sets with significant differences among components)</th>
<th>Field 1</th>
<th>Field 2</th>
<th>Field 1</th>
<th>Field 2</th>
<th>Field 3</th>
<th>Field 4</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupae on ground-litter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocks/Fields</td>
<td>0.1 (0)</td>
<td>0.2 (0)</td>
<td>0.3 (0)</td>
<td>1.4 (11)</td>
<td>0.0 (0)</td>
<td>0.4 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plots/B/F</td>
<td>3.6 (50)</td>
<td>1.2 (20)</td>
<td>2.9 (9)</td>
<td>3.4 (22)</td>
<td>0.0 (0)</td>
<td>2.2 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling sites/P/B/F</td>
<td>0.7 (0)</td>
<td>1.4 (20)</td>
<td>2.2 (9)</td>
<td>0.4 (0)</td>
<td>0.0 (0)</td>
<td>0.8 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subsamples/S/P/B/F</td>
<td>95.6 (-)</td>
<td>97.2 (1)</td>
<td>94.5 (-)</td>
<td>94.8 (-)</td>
<td>100.0 (-)</td>
<td>96.6 (-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pupae in plant-canopy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocks/Fields</td>
<td>3.8 (0)</td>
<td>7.1 (50)</td>
<td>2.0 (14)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>3.0 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plots/B/F</td>
<td>5.2 (25)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>1.5 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling sites/P/B/F</td>
<td>91.0 (-)</td>
<td>92.9 (-)</td>
<td>98.0 (-)</td>
<td>100.0 (-)</td>
<td>100.0 (-)</td>
<td>95.5 (-)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aGround litter results are means from NANOVA of 7, 6, 10, 11, 9 and 3 data sets (= weekly sampling periods) at fields 1 and 2, 1979, and fields 1-4, 1980, respectively (2688, 2304, 3264, 3264, 1728, and 576 total samples). Plant-canopy results are means from NANOVA of 4, 5, 2, 7, 5, and 1 data sets (256, 320, 128, 288, 160, and 32 total samples) at the same respective sites. Although 7, 6, 12, 13, 12, and 8 data sets actually were available, analyses were limited to dates when pupal densities were greater than 0.

bProbability of greater F value < 0.05.
Based on NANOVA results, simple random sampling plans should provide satisfactory pupal density estimates. Slight increases in precision might be gained by using 2-stage plans, but greater costs probably would offset these gains. These results also are consistent with the conclusions of Fedigo et al. (1972a) and Hammond and Fedigo (1976), who reported that GCW larvae are randomly dispersed in soybean fields.

Selection of sample-unit size

The optimal sample-unit size (i.e., no. 10 x 60-cm subunits/sampling site) was determined by solving

\[ n_{SUB}^{OPT} = \sqrt{\frac{C_{SUB}}{C_{SITE}}} \frac{S^2_{SITE}}{S^2_{SUB}} \]  

(Snedecor and Cochran 1976), where \( n_{SUB}^{OPT} \) is the optimal number of subunits, \( C_{SUB} \) and \( C_{SITE} \) are man-minute sampling costs required to examine a 10 x 60-cm subunit and to locate the next random sampling site, respectively, and \( S^2_{SUB} \) and \( S^2_{SITE} \) are estimates of variance among subunits and among sampling sites, respectively. Mean seasonal values of \( C_{SUB} \) and \( C_{SITE} \) were 2.125 and 1.56 man-min, respectively. Variance component estimates, determined by computing a NANOVA on data sets pooled from all 6 fields, were 0.05214 and 0.00153 for \( S^2_{SUB} \) and \( S^2_{SITE} \), respectively. The \( n_{SUB}^{OPT} \) estimate was 5.001, and thus five 10 x 60-cm subunits, or a 50 x 60-cm sampling unit, represents the best compromise between sampling precision and costs.
Determination of sample-size

The number of randomly located 50 x 60-cm samples required to estimate pupal densities with predetermined precision can be determined by solving

$$n_s = \frac{s^2}{c^2 \bar{x}^2}$$

(Karandinos 1976), where $n_s$ is the number of samples required, $s^2$ and $\bar{x}$ are sampling variance and mean, respectively, and $c$ is the desired precision level expressed by the standard error as a fraction of the mean. Because proper use of this equation requires that the underlying mathematical distribution be known, it often is more convenient to express $s^2$ as a function of $\bar{x}$:

$$n_s = f(\bar{x}) / c^2 \bar{x}^2$$

Two analyses were used to develop an appropriate functional relationship between the variance and mean for GCW pupal samples: Taylor's power law analysis (Taylor 1961), where $s^2 = \alpha \bar{x}^\beta$, and Iwao's mean crowding ($m$): mean ($m$) regression (Iwao 1968), where $s^2 = (\alpha + 1)\bar{x} + (\beta - 1)\bar{x}^2$. Both analyses explained all but a small portion of the variability between $s^2$ and $\bar{x}$ values (Table 5). Although either analysis provides a satisfactory functional equation to describe sampling variance, Taylor's power law procedure was selected because it had greater accountability, based on $R^2$ values, than the $m : m$ regression.

The slope coefficients from both Taylor's and Iwao's analyses can be interpreted as indices of population dispersion. Both procedures produced
Table 5. Results of Iwao's m:m regression and Taylor's power law analysis for GCW pupal samples in soybeans, Ames, IA, 1979-1980

<table>
<thead>
<tr>
<th>Analysis</th>
<th>no. obs.</th>
<th>Intercept (a)</th>
<th>Slope (b)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>m:m regression</td>
<td>46</td>
<td>0.0422</td>
<td>1.1972**</td>
<td>.82</td>
</tr>
<tr>
<td>Power law</td>
<td>46</td>
<td>1.2387**</td>
<td>1.0687**</td>
<td>.98</td>
</tr>
</tbody>
</table>

*Analyses computed on pooled 1979–1980 data. Each observation based on 32 or 64 weekly samples (50 x 60-cm sampling unit). Samples were taken on 58 weekly dates, 1979–1980, but pupae were recovered only on 46 dates.

* $p > t$-statistic < 0.05 for $H_o: \alpha = 0$ or $H_o: \beta = 1$.

** $p > t$-statistic < 0.01 for $H_o: \alpha = 0$ or $H_o: \beta = 1$.

$\beta$ terms slightly but significantly ($p < .05$) greater than 0, which indicates a small degree of aggregation among pupae. As discussed previously, ANOVA tests similarly detected occasional differences in pupal densities, particularly variation due to sampler effects. Therefore, although aggregation can be shown statistically, its biological basis and importance seem questionable. The $m:m$ intercept term also is an index of dispersion, and it identifies the basic components of a population. The relationship $\alpha = 0$ shows that GCW pupal populations exist as individuals rather than as colonies.
Substitution of the power law function into Equation 3 produced the formula

\[ n_s = \frac{\alpha x^{\beta-2}}{c^2}, \]

which was used to calculate sample-sizes for both mean and maximum pupal densities observed during 1979 and 1980 (Table 6). Under outbreak (high density) conditions in 1979, as few as twenty-four 50 x 60-cm samples would have been adequate to estimate peak pupal densities with 15% precision. In contrast, under endemic (low density) conditions in 1980, more than three times as many samples were required to achieve the same precision. Although Southwood (1978) recommended that 10% precision is needed for life table studies, approximately 900 samples (requiring 181 man-hours) would have been needed to estimate observed 1980 mean pupal densities with 10% precision. Obviously, sampling error greater than 10% must be accepted during low density conditions.

Because sample-sizes in Table 6 are calculated as a function of mean density, the mean must be known before sampling to determine \( n_s \). The 1979-1980 mean densities could be used, but many needless samples would be taken when densities were high. Sequential sampling procedures are more efficient, but because they simply classify populations into arbitrary density categories, the traditional sequential plans (e.g., Waters 1955) have little value for life table studies. Kuno (1969), however, derived a new sequential method, based on the \( m : m \) regression, which is well-suited for life table research. Kuno's method, designated a sequential count plan (Allen et al. 1972), estimates density with
Table 6. Fixed-size (n) sampling plans for estimating GCW pupal densities with 3 levels of precision during outbreak, endemic, and 1979–1980 average population levels

<table>
<thead>
<tr>
<th>Observed population level</th>
<th>No. samples (n) required for precision of</th>
<th>10%</th>
<th>15%</th>
<th>25%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbreak (1979)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum weekly density</td>
<td>54</td>
<td>24</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Mean seasonal density</td>
<td>190</td>
<td>85</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Endemic (1980)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum weekly density</td>
<td>175</td>
<td>78</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Mean seasonal density</td>
<td>892</td>
<td>397</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Average (1979–1980)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean density</td>
<td>392</td>
<td>174</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

\[ n = \frac{\alpha \beta^2}{C^2} \] where \( n \) = no. 50 x 60-cm samples required, \( \bar{x} \) = mean pupal density per sample, \( \alpha \) and \( \beta \) = intercept and slope terms from Taylor's power law analysis, and \( C \) = sampling precision expressed as \( \text{SE}/\bar{x} \).

\(^{b}\text{Maximum weekly mean of 32 or 64 samples.}\)
predetermined precision. Similar sequential count plans are proposed
here based on Taylor's power law analysis. Critical stop lines for these
plans were derived by substituting $\bar{X} = T_n/n$ into Equation 4, thus:

$$T_n = \left(\frac{(n^{1-\beta} \alpha)}{C^2}\right)^{1/(2-\beta)}$$

where $T_n$ = cumulative number pupae collected, $n$ = no. 50 x 60-cm samples
examined, $\alpha$ and $\beta$ are Taylor's coefficients, and $C$ is as defined pre­
viously.

Critical stop lines are given in Figure 5 for sampling GCW pupae
with 3 precision levels. Execution of these plans involves searching
randomly located 50 x 60-cm samples for pupae within the canopy and on
the litter, and successively plotting cumulative numbers of pupae col­
lected against sample number. Sampling continues until the stop line
designating the desired precision is crossed. Pupal density then can be
estimated at the predetermined precision level as $T_n/n$.

At extremely low pupal densities, the observed cumulative counts
may not exceed critical stop line values without an unreasonably large
sample size. A maximum of 80 samples/ha, requiring 16.25 man-hours,
was selected as the arbitrary end point. If cumulative pupal numbers
do not exceed critical $T_n$ values after 80 samples, density can be esti­
mated as $T_n/n$, but precision will be less than desired.
Fig. 5. Sequential count plans for sampling GCW pupae with 3 levels of precision
Evaluation of Alternative Sampling Procedures

**Fixed-time sampling**

Two-minute pupal samples were compared with intensive survey samples on the basis of 3 criteria: (1) precision returned per unit cost invested, (2) lack of biases in observed pupal distributions, and (3) fidelity to absolute pupal densities. Sampling precision and costs can be examined directly, but the relative net precision statistic (RNP) (Pedigo et al. 1972a) offers more meaningful evaluations of sampling efficiency. RNP is computed as \( \frac{1}{(C_s \bar{RV})}(100) \), where \( C_s \) is the cost (man-hours) of locating and searching 1 sample, and \( \bar{RV} \) (relative variation) is the mean seasonal sampling precision expressed by the standard error as a percent of mean density. The larger the RNP statistic, the greater the efficiency in terms of precision returned for sampling effort expended. RNP values for 2-minute and intensive-survey samples were 61.1 and 29.5, respectively. Although intensive samples provided approximately twice the precision of a 2-minute sample (\( \bar{RV}_{\text{intensive}} = 14.2 \) vs. \( \bar{RV}_{2\text{-min}} = 27.6 \)), the intensive samples also required slightly more than 4 times the man-hours. Overall, intensive samples were less than half as efficient as fixed-time samples.

Chi-squared analyses were computed for each sampling period to detect procedural biases in pupal distributions among plant-canopy and soil-litter sites. The proportions of pupae collected from each site did not differ significantly (\( p < .05 \)) between 2-minute and intensive survey samples. A single exception to these results occurred in field 1 on August 7, when 2-minute sampling recovered significantly fewer pupae than
expected from soil-litter locations. Maximum seasonal pupal densities (8 pupae/m²) also were recorded on that date, which suggests that fixed-time samples are least reliable when pupal densities are high.

Fidelity was examined with correlation and linear-regression analyses. Mean density estimates from 2-minute samples agreed closely with absolute estimates from intensive surveys (r = .86, p < .05). Linear regression of 2-minute means on intensive-survey means suggests that, given some refinements, the relative density estimates from fixed-time samples could be converted reliably to absolute population estimates. The regression model for this conversion is $y = 0.1163 + 0.3724x$ ($n = 6$, $R^2 = .75$), where $y$ is the 2-minute mean density estimate and $x$ is the absolute population. Although these analyses are based on only 6 weekly sampling periods, they indicate that fixed-time samples are a potential alternative to intensive survey procedures.

**Single-date sampling**

Single-date samples were converted directly to $l_x$ and $100S_x$ life table estimates and compared with intensive-survey results (Table 7). The estimated $l_x$ and $100S_x$ values are mean numbers of total pupal exuviae collected (expressed on a per ha basis) and the proportion of this total classified as having emerged, respectively. Intensive survey samples were integrated into the actual $l_x$ values by using the area-under-the-curve method (Southwood 1978). Actual $100S_x$ values were calculated as $(pupal l_x - moth l_x)/pupal l_x$, where moth $l_x$ terms were determined by graphical summation of GCW moth-flushing samples as described by Pedigo (1980).
Table 7. GCW pupal life-table values calculated from samples taken at successive weekly intervals (actual values) and from single-date samples taken at the end of pupation (estimated values)

<table>
<thead>
<tr>
<th>Site</th>
<th>Actual</th>
<th>Estimated</th>
<th>% error</th>
<th>Actual</th>
<th>Estimated</th>
<th>% error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. live pupae/ha ( (l_x) )</td>
<td></td>
<td></td>
<td>% survivorship ( (100 S_x) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>110703</td>
<td>29080</td>
<td>- 74</td>
<td>2.5</td>
<td>83.6</td>
<td>+ 3244</td>
</tr>
<tr>
<td>Field 2</td>
<td>43872</td>
<td>20833</td>
<td>- 52</td>
<td>6.4</td>
<td>83.3</td>
<td>+ 1202</td>
</tr>
<tr>
<td>1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>3813</td>
<td>434</td>
<td>- 89</td>
<td>8.2</td>
<td>100.0</td>
<td>+ 1120</td>
</tr>
<tr>
<td>Field 2</td>
<td>4336</td>
<td>3039</td>
<td>- 30</td>
<td>18.5</td>
<td>71.4</td>
<td>+ 286</td>
</tr>
<tr>
<td>Field 3</td>
<td>1789</td>
<td>434</td>
<td>- 76</td>
<td>---b</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Field 4</td>
<td>1280</td>
<td>3472</td>
<td>+171</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1980</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field 1</td>
<td>10575</td>
<td>9549</td>
<td>- 10</td>
<td>7.2</td>
<td>18.1</td>
<td>+ 151</td>
</tr>
<tr>
<td>Field 2</td>
<td>45858</td>
<td>24306</td>
<td>- 50</td>
<td>2.8</td>
<td>46.4</td>
<td>+ 1557</td>
</tr>
<tr>
<td>Field 3</td>
<td>40900</td>
<td>22569</td>
<td>- 45</td>
<td>---b</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Field 4</td>
<td>755</td>
<td>2604</td>
<td>+245</td>
<td>---b</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\% error = [(estm. - actual)/actual] 100.\)

\(^bActual 100S_x values not determined at fields 3 and 4.\)

\(^cPupal densities below detectable levels during gen. 2, 1979.\)
In general, single-date sampling seriously underestimated pupal $l_x$ values (Table 7). Several factors contributed to this error. First, because GCW pupal exuviae weather rapidly in the field, remains from the initial individuals of the generation deteriorated beyond recovery by the time samples were taken (5 to 8 wk later). Second, actual $l_x$ values include both prepupae and pupae. Any prepupae that failed to develop to the pupal stage would not have left any identifiable remains for single-date sampling. The $l_x$ values were overestimated only at field 4, 1980. Densities were extremely low at this site ($\bar{X} = 1$ pupa/18 m$^2$) and were subject to large sampling errors. Therefore, both actual and estimated $l_x$ values are of doubtful reliability and may not represent actual population trends.

Single-date sampling grossly overestimated pupal survivorship (Table 7). Many pupal exuviae were collected as incomplete fragments and were difficult to assign a definite fate. Obviously, determinations were biased against the mortality categories. Based on the errors in estimated $100s_x$ and $l_x$ values, it is clear that single-date sampling has little value for GCW life-table research. Although these plans offer considerable savings in manpower sampling costs, it seems they are suited better for insects that construct cocoons with longer residual field lives.
PART III: GREEN CLOVERWORM POPULATION DYNAMICS:

PUPAL LIFE TABLE STUDIES IN IOWA SOYBEAN
ABSTRACT

Six partial life tables were prepared for pupal green cloverworms *Plathypena scabra* (F.), during 1979 and 1980. Studies complemented concurrent larval and adult life table research.

Pupal dynamics were characterized by large 1st generation densities that declined below detectable levels during generation 2 in 1979, and by small 1st generation densities that expanded 7-fold during generation 2 in 1980. The 1979 and 1980 density patterns are characteristic of outbreak and endemic GCW population configurations, respectively. Pupal survivorship was 4-fold greater under endemic population levels than under outbreak levels during generation 1.

Pupae were parasitized by 11 primary species, but *Vulgichneumon brevicinctor* (Say) (Hymenoptera: Ichneumonidae) and *Winthemia sinuata* Reinhard (Diptera: Tachinidae) consistently accounted for most parasitism. Total parasitization represented less than 18% of total 1979 pupal mortality but accounted for 60% of total 1980 mortality. Infections caused by a microsporidan and the entomogenous fungus, *Nomuraea rileyi* (Farlow) Sampson, accounted for a small portion of total mortality, as did deaths attributed to nonviability. Predation was an important source of pupal mortality and was 2.5 times greater under outbreak levels than under endemic levels during generation 1 (i.e., 64% mortality vs 24.1% mortality).

A modified key factor analysis indicated that no single mortality component was correlated with changes in total pupal mortality. Regression analyses generally failed to detect density-dependent mortality.
factors, but did indicate that *V. brevicinctor* acted in a delayed density-dependent manner and that total pupal mortality was density-dependent.
INTRODUCTION

The green cloverworm (GCW), Plathypena scabra (F.), is a common U.S. soybean pest that sporadically causes serious defoliation in the Midwest. Because data on the mechanisms of population regulation are essential to develop effective pest suppression programs, GCW life table studies were begun in Iowa during 1977. Initially, studies concentrated on the larvae and adults because sampling procedures were well-understood for those stages. Furthermore, previous research had indicated that pupal mortality was minimal and that larval mortality accounted for a large portion of total GCW mortality (Pedigo et al. 1972b).

Pupal dispersion and sampling studies (Parts I and II of this dissertation) permitted expansion of the GCW life table effort to include the pupal stage during 1979 and 1980. This paper presents the results of those studies and includes analyses to assess the significance and density relationships of pupal mortality components.
METHODS AND MATERIALS

Intensive Pupal Sampling Program

GCW pupal surveys were coordinated with larval and adult life table studies in 2 soybean fields during 1979 and in 4 fields during 1980. Field descriptions, sampling design, sample-unit size, sample size, and number of sampling periods were as described in Part I of this dissertation.

All pupae collected were placed in environmental chambers (27°C, 76% RH) for observation. Parasitoids were identified by Dr. E. E. Grissell, USDA Systematic Entomology Laboratory, and by Dr. J. W. Mertins, Department of Entomology, Iowa State University. Pupae that failed to complete development after accumulating 1200 $T^*_U$ (>5 times normal requirement for pupal development) (Hammond et al. 1980) were dissected for evidence of parasitism. Histopathological examinations were performed by specialists at the Department of Entomology, Iowa State University. Examinations included tissue smears (1 wet mount and 1 heat fixed, Giemsa-stained slide) and culture streak plates (1 nutrient agar and 1 Saubourod's dextrose agar with 1% yeast extract) of each specimen. Particular attention was given to detection of Nomuraea rileyi (Farlow) Sampson. This entomogenous fungus plays a key role in larval GCW population regulation but its impact on GCW pupae is unstudied.

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Intensive Moth Surveys

Adult density estimates were required to calculate pupal survivorship. Moths were sampled with the flushing procedure developed by Pedigo et al. (1982). Flush samples were taken within 1-ha plots adjacent to the pupal study areas at fields 1 and 2, 1979 and 1980. Field design, sample-unit size, sample-size, and sampling mechanics were as described by Pedigo et al. (1982).

Preparation of Partial Life Tables

The area-under-the-curve method (Southwood 1978) was used to integrate pupal and moth count-data into $l_x$ values. Moth $l_x$ calculations were as described by Pedigo. Pupal $l_x$ calculations were based on a mean pupal developmental time of $218 \ T_u$ which was estimated from the data of Hammond et al. (1979) by using a 12.2°C threshold temperature. Pupal $d_x$ terms were determined by subtracting moth $l_x$ from pupal $l_x$. The incidence of parasitism, disease, and nonviability was determined from the fates of pupae held in environmental chambers.

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RESULTS AND DISCUSSION

Density and Survivorship Trends

Partial life tables were prepared for GCW pupal populations at each soybean field, and these were averaged to permit comparison of overall seasonal differences in pupal dynamics. Striking differences were observed in GCW pupal densities between 1979 and 1980 (Tables 8 and 9). The 1979 populations were large during generation 1 (ca. 8 pupae/m²) but then declined below detectable levels during generation 2. In contrast, 1980 pupal densities were small during generation 1 (< 0.5 pupae/m²) but increased almost 7-fold during generation 2. These contrasting trends are typical of outbreak and endemic GCW population configurations, respectively, according to a hypothesis of GCW dynamics proposed by Pedigo. Endemic populations expand several-fold from generation 1 to generation 2, but overall densities remain low. Outbreak populations are much larger during generation 1 and occasionally exceed economic-injury levels, but then decline drastically during generation 2. Population collapse is caused by epizootics of N. rileyi, which reduce survivorship of large larvae during generation 1 and of small larvae during generation 2. Based on larval samples, Pedigo categorized GCW populations as outbreak during 1979 and as endemic during 1980. Pupal density trends agreed completely with the previously reported larval trends and are further evidence in support of the GCW population dynamics hypothesis.

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Table 8. Partial life tables for 1st and 2nd generation GCW pupae in soybeans, Ames, IA 1979 (no./ha)\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>(x)</th>
<th>(l_x)</th>
<th>(d_F)</th>
<th>(100q_x)</th>
<th>(S_x)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Generation 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large larvae (stages 5 &amp; 6)</td>
<td>99,236</td>
<td></td>
<td>22.1</td>
<td>.779</td>
<td></td>
</tr>
<tr>
<td>Prepupae and pupae</td>
<td>77,288</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasitization</td>
<td>17.5</td>
<td>.825</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. sinuata</td>
<td>3.4</td>
<td>.966</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. hyphantriae</td>
<td>2.4</td>
<td>.976</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>tachinid complex</strong> \textsuperscript{b}</td>
<td>2.7</td>
<td>.973</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. brevicinctor</td>
<td>8.0</td>
<td>.920</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Coccyygymimus spp.</strong> \textsuperscript{c}</td>
<td>.2</td>
<td>.998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. aequalis</td>
<td>.2</td>
<td>.998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. pertubatrix</td>
<td>.2</td>
<td>.998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. sexdentatus</td>
<td>.2</td>
<td>.998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>other</strong> \textsuperscript{d}</td>
<td>.2</td>
<td>.998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td>9.3</td>
<td>.907</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>microsporidan</td>
<td>2.0</td>
<td>.980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. rileyi</td>
<td>6.3</td>
<td>.937</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonviability\textsuperscript{e}</td>
<td>6.6</td>
<td>.934</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predation\textsuperscript{f}</td>
<td>64.0</td>
<td>.360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td>96.4</td>
<td>.036</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moths</td>
<td>2,778</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Generation 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large larvae</td>
<td>542</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepupae and pupae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moths</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Means of fields 1 and 2, 1979.
\textsuperscript{b}Larvae that were not identified reliably.
\textsuperscript{c}Indistinguishable males of C. aequalis, C. annulipes, and C. nudus.
\textsuperscript{d}Larval hymenopteran; probably V. brevicinctor.
\textsuperscript{e}Pupae that failed to complete development after 1200 \(T_u\) and that did not exhibit evidence of parasitism or disease.
\textsuperscript{f}Residual mortality not accounted for by parasitism, disease, and non-viability.
\textsuperscript{g}Densities below detectable level.
Table 9. Partial life tables for 1st and 2nd generation GCW pupae in soybeans, Ames, IA, 1980 (no./ha)\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Generation 1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x)</td>
<td>(1_x)</td>
<td>(d_x)</td>
<td>(100q_x)</td>
</tr>
<tr>
<td>Large larvae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(stages 5 &amp; 6)</td>
<td>7,102</td>
<td></td>
<td>42.6</td>
<td>.574</td>
</tr>
<tr>
<td>Prepupae and pupae</td>
<td>4,074</td>
<td>Parasitization</td>
<td>51.3</td>
<td>.487</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W. sinuata</td>
<td>16.2</td>
<td>.838</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. hyphantriae</td>
<td>2.7</td>
<td>.973</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tachinid complex\textsuperscript{b}</td>
<td>5.4</td>
<td>.946</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V. brevicinctor\textsuperscript{c}</td>
<td>21.6</td>
<td>.784</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. aequalis</td>
<td>2.7</td>
<td>.973</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. trifasciata</td>
<td>2.7</td>
<td>.973</td>
</tr>
<tr>
<td>Disease</td>
<td>0.0</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonviability</td>
<td>10.8</td>
<td>.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predation\textsuperscript{e}</td>
<td>24.2</td>
<td>.758</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTALS</td>
<td>86.3</td>
<td>.137</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                | Generation 2 |
|                | \(x\) | \(1_x\) | \(d_x\) | \(100q_x\) | \(S_x\) |
| Large larvae   | 55,782 | Parasitization | 49.5 | .505 |
| Prepupae and pupae | 28,216 | W. sinuata | 17.5 | .825 |
|                |       | B. hyphantriae | 2.1 | .979 |
|                |       | O. assimilis | .4 | .996 |
|                |       | tachinid complex\textsuperscript{b} | 3.3 | .967 |
|                |       | V. brevicinctor\textsuperscript{c} | 28.3 | .717 |
|                |       | Coccygomimus spp.\textsuperscript{f} | 1.2 | .988 |
|                |       | C. aequalis | .8 | .992 |
|                |       | C. nudus | .4 | .996 |
|                |       | B. ovata | 2.9 | .971 |
| Disease        | .4  | .996 |
| N. rileyi      | .4  | .996 |
| Nonviability   | 6.7  | .993 |
| Predation\textsuperscript{e} | 32.2 | .678 |
| TOTALS         | 96.4 | .036 |

\(\textsuperscript{a}\)\(x\) values are means of fields 1 and 2, 1980; \(d_x\) column based on pupal samples at fields 1-4, 1980.

\(\textsuperscript{b}\)Larvae that were not identified reliably.

\(\textsuperscript{c}\)Includes 5.4% and 0.8% hyperparasitism of V. brevicinctor by E. tachinae during generations 1 and 2, respectively.

\(\textsuperscript{d}\)Pupae that failed to complete development after 1200 \(T_U\) and that did not exhibit evidence of parasitism or disease.

\(\textsuperscript{e}\)Residual mortality not accounted for by parasitism, disease, and nonviability.

\(\textsuperscript{f}\)Indistinguishable males of C. aequalis, C. annulipes, and C. nudus.
Large differences similarly occurred in pupal mortality between years. During generation 1, survivorship was approximately 4 times greater under 1980 endemic population levels than under outbreak conditions in 1979. Even during the endemic year, however, 86.3% of the 1st generation pupae died. During the 2nd generation in 1979, mortality equalled 1st generation outbreak levels. Density and survivorship data suggest that overall pupal mortality acts in a density-dependent manner.

Pupal mortality levels recorded in Tables 8 and 9 were considerably greater than had been suspected. Earlier studies with experimental GCW cohorts showed that pupal mortality was minimal (Pedigo et al. 1972b), and it was assumed that deaths of large larvae (stages 5 and 6) accounted for most mortality between the late larval and adult stages. Pupal mortality, however, actually accounted for at least 50% of late larval to adult mortality during 1980 and for 77% during 1979. It seems that pupal mortality is much greater and that large larval mortality is much less than believed previously.

Factors responsible for pupal deaths also were unexpectedly diverse. The natural enemy complex included 11 species of primary parasitoids: Blondelia hyphantriae (Tothill), Oswaldia assimilis (Townsend), and Winthemia sinuata Reinhard (Diptera: Tachinidae); Pediobius sexdentatus (Girault) (Hymenoptera: Eulophidae); Colpotrochia (Colpotrochia) trifasciata (Cresson), Rubicundiella perturbatrix Heinrich, Coccygomimus aequalis (Provancher), C. annulipes (Brulle), C. nudus (Townes), and Vulgichneumon brevicinctor Say (Hymenoptera: Ichneumonidae); and Brachymeria ovata (Say) (Hymenoptera: Chalcididae). With the exception
of the 3 tachinids (Lentz and Pedigo 1975) and *V. brevicinctor* (Krombein et al. 1979), none has been reported previously from GCW. Comparison with larval GCW parasitism records from Iowa (Lentz and Pedigo 1975) shows that the pupal parasitoid complex consists of more species than the complex for all 6 larval stages combined. In addition to these primary species, a hyperparasitoid, *Eupteromalus tachinae* Gahan (Hymenoptera: Pteromalidae), occasionally was reared from pupae parasitized by *V. brevicinctor*.

Of the primary parasitoids, the 1st six (*B. hyphantriae* through *R. perturbatrix*) are larval-pupal parasitoids and the remainder directly attack the pupal stage. *C. trifasciata* and the *Coccymomimus* spp. complex typically parasitize lepidopteran pupae in the heavy undergrowth and ground cover of mesophytic deciduous forests (Townes and Townes 1959, 1960). Their presence in soybean fields located many miles from such forested sites suggests that these species are preadapted for establishment in agronomic ecosystems.

Tables 8 and 9 show that both parasitoid diversity and impact were greater under endemic conditions in 1980 than under outbreak conditions in 1979. Parasitization accounted for approximately 60% of total 1980 pupal mortality, compared to < 18% in 1979. During each year, *W. sinuata* and *V. brevicinctor* consistently accounted for the majority of total parasitism. Lentz and Pedigo (1975) similarly reported that *W. sinuata* is among the most frequently reared GCW larval parasitoids in Iowa. The remaining pupal parasitoids were reared much less frequently (e.g., *P. sexdentatus*, *R. perturbatrix*, and *C. trifasciata* each were reared only once).
Histopathological examinations yielded many bacteria and fungi from pupal cadavers, but most were secondary, post mortem contaminants. Two agents, however, produced fatal infections: a microsporidan tentatively identified as *Vairimorpha* sp., and the fungus, *N. rileyi*. The microsporidan has not been isolated previously from natural GCW populations, although Henry (1981) reported that GCW are susceptible to infection by *Vairimorpha necatrix* (Kramer). Further pathogenicity tests are planned to identify the agent involved.

Tables 8 and 9 show that the microsporidan produced only minimal mortality during 1979 and no detectable mortality in 1980. However, the signs and symptoms of pupal infection, which included incomplete or weak sclerotization, the presence of microsporida in fat body, and failure of moths to completely break eclosion sutures and emerge from pupal exuviae, suggest that debilitative, sublethal infections may be important and should be investigated. Additional research is needed to determine mode of infection and incidence in egg, larval, and adult stages, and to explain the absence of microsporidan-caused deaths in 1980.

As noted earlier, *N. rileyi* produces dramatic epizootics and population crashes among outbreak GCW larvae. GCW pupae also are killed by *N. rileyi*, especially during outbreak years, but the incidence of pupal infection is much less than among larvae and is not associated with population collapse. Further, it seems likely that pupal deaths caused by *N. rileyi* result indirectly from larval infection rather than directly through pupal infection. Although pupae seem less susceptible to *N. rileyi* than larvae, they may play an important role in maintaining
innoculum. Fungal-infected cadavers resist weathering, and it seems likely that pupal cadavers protect *N. rileyi* from desiccation, ultraviolet light, and similar adverse environmental factors. For example, pupae stored in the laboratory for up to two years contained viable spores that germinated, grew vigorously, and sporulated on artificial media.

A small proportion of the pupae held in environmental chambers died from unknown factors and were designated nonviable. This category undoubtedly included natural factors, such as inherent unfitness, and abnormal artifacts, such as mechanical injury at collection or improper laboratory conditions for development. Prepupae especially were susceptible to physical damage. The proportion of nonviability was similar between years.

Residual mortality remaining in life tables after accounting for parasitism, disease, and nonviability was attributed to predation. Although this mortality category actually included losses from all sources that could not be measured directly in the laboratory, such as deaths caused by adverse field environmental conditions, survivorship studies with experimental cohorts (Bechinski et al. 1982) support the contention that predation is the primary component. Tables 8 and 9 show that predation was a major source of pupal mortality during 1979 and 1980, but was particularly significant under outbreak GCW population conditions.

**Detection of key factors**

Varley et al. (1973) proposed an analytical technique, termed key factor analysis, which determines the contributions of mortality factors
to total population change. The analysis is based on the relationship

\[ K = k_1 + k_2 + k_3 + \ldots k_n \]  \hspace{1cm} (6)

where \( k_n \)-values are logarithmic measures of the killing power of successively acting mortality factors and \( K \) is total mortality, usually during a generation. The \( k \)-value that contributes most to variation in \( K \) is designated the key factor most associated with changes in survivorship.

Although key factors usually are studied in relation to overall population trend, identification of key factors for an age class can be more informative (Meats 1971). A pupal GCW key factor analysis based on Equation 12 (where \( K \) = total pupal mortality and \( k \)-values = killing power of each mortality agent) is inappropriate because pupal mortality factors do not act in succession but instead overlap in time. Individual \( k \)-values can be calculated, but they are nonadditive (Beaver 1965). An essentially similar analysis was computed, however, by using life tables \( 100q_x \) terms:

\[ 100q_x = 100q_{x1} + 100q_{x2} + 100q_{x3} + \ldots 100q_{xm} \]

where \( 100q_x \) = total generation pupal mortality and \( 100q_{xm} \) values = individual pupal mortality components. Mortality values from the original 6 partial life tables were evaluated graphically as suggested by Varley et al. (1973) (Fig. 6). Because these visual evaluations failed to identify an obvious key factor associated with changes in overall mortality, a series of correlation and regression procedures were conducted by using the methods of Podoler and Rogers (1975). Neither statistical
Fig. 6. Seasonal fluctuations in GCW pupal density \((\log l_x)\), total pupal mortality \(100 Q_x\), and pupal mortality subcomponents \((100q_{xm} \text{ values})\)

Plot-yr. 1 = field 1, 1979; 2 = field 2, 1979; 3 = field 1, 1980, generation 1; 4 = field 1, 1980, generation 2; 5 = field 2, 1980, generation 1; and 6 = field 2, 1980, generation 2.
PUPAL MORTALITY COMPONENTS (100q_x^n values)

100 Q_x log l_x

Plot-years

PUPAL TOTAL MORTALITY

LARGE LARVAL MORTALITY
method, however, revealed the existence of a key factor. It is plausible, however, that 2 different components function as key factors, 1 restricted to endemic populations and the other limited to outbreak types. Hassel and Huffaker (1969), for example, detected 2 key factors in populations of the Mediterranean flour moth, Anagasta kuehniella (Zeller), with 1 factor detectable only during the initial 12 generations and the other only during later generations. Further studies under endemic and outbreak conditions are needed to determine if a similar situation occurs among pupal GCW populations.

Density relationships

Information on how mortality factors function in relation to host density is a prerequisite to understanding population regulation. Functional relationships can be examined by calculating regressions between percent mortality and population density, but Varley et al. (1973) suggest that a better approach is to regress individual k-values against the logarithm of the population density on which mortality acted. Therefore, GCW pupal $S_x$ terms for every mortality factor in the original 6 partial life tables were converted to k-values and regressed against $\log_{10} l_x$ values. Regression coefficients from these analyses did not differ statistically from 0, which suggests that pupal mortality components acted in a density-independent manner. Although detection of density-dependent mortality had been expected, Southwood (1978) aptly observed that density dependence often is difficult to demonstrate from census data and that failure to detect such factors does not prove their absence.
Latheef et al. (1979), for example, similarly failed to demonstrate density-dependent mortality during the egg, larval, and pupal stages of the alfalfa weevil, Hypera postica (Gyllenhal).

Because k-values for many mortality components were small and varied little between years, these factors can be assigned constant values for practical purposes. A possible exception was mortality caused by the parasitoid, V. brevicinctor. When data points for this agent were linked serially in time sequence (Fig. 7), plots from 4 sites tended to show elements of the anticlockwise spiralling typical of delayed density-dependent mortality agents (Varley et al. 1973). Proofs of such relationships are difficult from field data, and further data on V. brevicinctor densities and levels of pupal parasitization are needed to properly assess host/parasitoid relationships.

Although density dependence was not detected among individual mortality factors, regression of total pupal mortality (K-values) on log density produced a slope coefficient significantly greater than 0, which suggests that overall pupal mortality was density dependent. The regression model was $Y = -0.7347 + 0.4578x$ ($r^2 = 0.74$, $n = 6$), where $Y =$ total pupal mortality expected (expressed as killing power) and $x = \log_{10}$ density. Although these results seem contrary to previous results from individual k-value regressions, Varley and Gradwell (1970) reported that the additive effects of several statistically insignificant density-dependent mortality factors can produce a significant cumulative relationship. Thus, it seems likely that several individual mortality factors functioned in a direct, albeit weak, density-dependent manner. Because
Fig. 7. Relationship between V. brevicinctor k-values (killing power) and GCW pupal density.

Each data point is the mean from a weekly sampling period. Arrows join data points in a time series, where A = field 1, 1979, B = field 2, 1979, C = field 4, 1980, and D = field 5, 1980.
V. brevicinctor k-value (killing power)

Log₁₀ pupal density/ha

A

B

C

D
it is widely accepted that density-dependent mortalities regulate or stabilize populations (Varley and Gradwell 1970), the pupal stage may play an important regulatory role in GCW population dynamics.
SUMMARY AND CONCLUSIONS

The green cloverworm (GCW), *Plathypena scabia* (F.), is considered the primary insect pest of Iowa soybean. Larval and adult GCW life table studies have been conducted in Iowa soybean since 1977 to develop the data base required to design management strategies for this pest. Although this approach has produced considerable advances in understanding GCW population changes, the lack of mortality data for the remaining life stages (i.e., eggs and pupae) prevents a complete overview of pest dynamics. Research reported here was an attempt to fill this data gap with respect to the pupal stage.

Research was directed simultaneously toward 2 basic objectives, the primary being to quantify pupal survivorship by using the life table approach. Life table studies required, however, accurate pupal density estimates. Therefore, the development of suitable sampling techniques and programs received much attention.

Sampling-plan development required information on pupal spatial patterns and these data were obtained by examining pupal microspatial distribution in 6 soybean fields during 1979 and 1980. Approximately 90% of the pupae were located on the soil surface, with the remaining 10% within the plant canopy. In the former sites, biases were detected in pupal densities with respect to directional aspect and distance from the row. In the latter sites, biases were observed in densities with respect to plant part and height within the canopy. Soil litter cover and pupal population density contributed to biases. These microspatial
data provided the basis for selecting the proper sampling universe and sample-unit.

A sampling program suitable for pupal life table studies was developed. The program incorporates a sequential count plan (Allen et al. 1972) that was derived from Taylor's power law (Taylor 1961). Execution of the plan in the field involves locating random sampling sites (50-cm long x 60-cm wide, centered over the soybean row) within a 0.8-ha study area in a soybean field and searching plants and soil surface for pupae. Sampling continues until the cumulative number of pupae collected exceeds the calculated stop lines for the precision level desired. This count plan is well-suited for life table research because it provides density estimates with predetermined precision for the absolute minimum sample size.

Two sampling techniques, fixed-time and single-date methods, were evaluated as labor-saving alternatives to the intensive pupal sampling program. The former technique compared favorably with the intensive program in terms of precision and cost, and the latter method showed no potential for use in GCW pupal life table research.

Six partial life tables were constructed for GCW pupae during 1979 and 1980. Studies showed that pupal density trends conformed to outbreak and endemic population configurations during 1979 and 1980, respectively. Total mortality ranged from 86.3 to 96.4% each generation, and was much greater than previously suspected. Parasitism (11 primary parasitoid species), disease (2 pathogens), nonviability, and predation were identified as causes of pupal mortality. No single mortality component
acted as a key factor (Varley and Gradwell 1970), nor could direct density
dependence be detected among individual mortality components. Overall
pupal mortality was directly density dependent and seems to represent the
cumulative effects of several statistically insignificant but biologically
important direct density dependent components.

Although these pupal sampling and life table studies have advanced
the knowledge of GCW dynamics, they also identified several areas for
subsequent research. In particular, the role of pupae infected by the
fungus, *N. rileyi*, should be investigated to determine how infected
pupal cadavers contribute to the maintenance of conidia between larval
generations. Similarly, little is known about the microsporidan recovered
from pupae. Identification of the specific agent involved is an immediate
need. Future studies also should examine the role of lethal and sub-
lethal infections in other GCW life stages. Another area for future
studies concerns the actual utilization of pupal natural enemies in
management programs. Although the conservation and manipulation of
pupal pathogens, parasitoids, and predators hold promise as components
of a GCW management system, factors influencing natural enemy impact
deserve detailed examination. For the majority of parasitoid species,
even the most basic biological data are lacking. Obviously, many fruit-
ful areas remain for future study.
LITERATURE CITED


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