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Differences in the Diapause Response of Boll Weevils from the High Plains and Central Texas and the Significance of this Phenomenon in Revising Present Fall Insecticidal Control Programs

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SUMMARY

Boll weevils on the High Plains of Texas enter diapause considerably earlier in the fall and in greater percentages than do those in Central Texas. This difference in response is due not only to differences in environment between the two areas, namely, earlier occurring more severe winters in the High Plains than in Central Texas, but also to a difference in the genetic makeup of the two weevil populations. The difference in genotype was found when the weevils were maintained in identical photoperiods. Short days induce diapause in both populations; however, significantly greater percentages of the High Plains weevils entered diapause under inductive photoperiods than did those from College Station or Presidio. It was concluded that the boll weevil is becoming adapted to the environment of the High Plains and a distinctive photoperiodic race of the species may have already evolved in this area.

It is suggested that a new type of approach for reducing diapausing boll weevils might be particularly effective in the High and Rolling Plains. This approach would have two phases. Phase 1 would be directed towards killing the last reproductive generation of adult females before they lay the eggs that give rise to the potentially overwintering adults. This would be accomplished with insecticidal applications made at 5-day intervals during September. Phase 2 would have as its objective the killing of any diapausing adults in October that may have survived earlier insecticidal treatments or that may have developed from eggs laid between applications in September.

INTRODUCTION

THE BOLL WEEVIL presently is expanding its geographical distribution both southward and northward. It is suspected that the center of origin of the boll weevil was Mexico or Central America. The pest has recently entered Colombia and Venezuela to the south. Invasions have occurred to the north into the El Paso Valley and the High Plains of Texas and into the Sonora area of Northwestern Mexico and Arizona. These recent invasions should be sufficient evidence to point out the adaptability of the species to great differences in environment as found at these widely separated geographical locations. The ability to diapause is evidently one of the mechanisms used by the weevil to expand its range of distribution.

There may be considerable differences among widely separated insect populations in the nature of the diapause response to certain environmental stimuli such as photoperiod. Local variations in the characteristics of the diapause response may operate as important isolating mechanisms in the formation of geographical races and possibly in the development of new and distinct species.

At the present time, insecticidal control programs are being directed at fall populations of boll weevils in the Presidio-El Paso Valley and the High and Rolling Plains area of Texas. These control programs have as their objective the killing of the potentially overwintering populations before they leave the cotton fields for hibernating sites. The present experiments were designed to detect any basic differences in the diapause response of boll weevil populations from different geographical locations. This information is essential to the development of more efficient control programs. The knowledge of when diapause occurs in field populations and the environmental conditions which trigger the diapause is absolutely essential in determining when the insecticidal applications should be initiated to prevent the formation of potential overwintering populations.

MATERIALS AND METHODS

Source of Boll Weevils

Frequent collections of adult boll weevils and infested squares were made during the fall of 1964 in cotton fields near College Station and near the junction of the High and Rolling Plains in the vicinity of Afton, Texas (Dickens County). The seasonal incidence of diapause was determined in the weevils taken in these collections. In addition, for photoperiodic studies, adult weevils were collected from the two above locations and Presidio and cultured in the laboratory. The laboratory cultures were maintained on a cottonseed meal diet according to procedures described by Sterling *et al.* (1965). Henceforth in this paper the various populations will be designated as follows: College Station (CS), Afton (HP) and Presidio (PD).

Handling of Weevils

Since procedures for handling the weevils varied for each experiment, they will be presented in the appropriate section of "Results."

Determination of Diapause

The diapause status of adult weevils were determined according to the method outlined by Brazzel and Newsom (1959). The adults were dissected under the microscope, the appearance of the fat body was noted and size of the ovaries or testes measured. Adults were classified as being reproductive, "intermediate-diapause," or "firm-diapause" according to the criteria furnished by Brazzel and Newsom (1959).

Photoperiod Studies

All life history stages of the boll weevils were maintained in bioclimatic cabinets programmed to maintain selected photoperiods at 70° F. The photoperiods were programmed by time switches wired to 3 daylight type 15 watt fluorescent lamps in each cabinet. The timers were set to provide the desired number of hours of light and dark per day.

RESULTS AND DISCUSSION

Seasonal Incidence of Diapause in HP and CS Populations

A. *Occurrence of diapause in field-collected adult boll weevils examined on date of collection.* Most authors who have studied diapause in field populations of the boll weevil have reported data obtained by examination of adults shortly after the insects had been collected (Brazzel and Newsom, 1959; Brazzel and Hightower, 1960; Lloyd and Merkyl, 1961; Beckham, 1963; Lloyd et al., 1964; Mitchell and Mistric, 1965).

A similar type study was made during the present investigation. Adult boll weevils were collected at random in cotton fields near Afton (HP) and College Station (CS). These insects were brought to the laboratory, dissected, examined under a microscope and their diapause status determined according to procedures described earlier in this report.

Percentages of diapausing weevils were small in the collections from both populations prior to early September (Figure 1). Thereafter, the incidence of diapausing weevils gradually increased as the season advanced. However, the percentages of diapausing adults varied greatly between collection dates with the HP population having more variance than the CS one. For example, in the HP collection of September 15 there were only 12 percent diapausing adults. Five days later, the HP collection contained 96 percent adults in diapause, an increase of 84 percent. Near the end of September, the percentage of diapausing weevils had decreased to approximately 20 percent. The CS collection showed a similar type of variability but not to such a great extreme. The extreme variability between successive collection dates is difficult to explain. Perhaps, during the fall, migrations of reproductive and diapausing weevils from field to field and from cotton fields to nearby overwintering sites might be responsible for some of this difference. Also, there may be inherent errors in this method of sampling since it does not take into account the age structure of the population or differences in behavior between reproductive and diapausing weevils.

The diapause curves plotted in Figure 1 clearly show that the incidence of diapause after early September generally was considerably greater in the HP than in the CS population. As the season advanced the number of diapausing weevils in both populations increased; however, the CS population lagged several days behind the HP weevils in this respect. It should be expected that the differences in the occurrence of diapause between the two populations will vary according to seasonal differences in climate between the High Plains and College Station.

In the northern hemisphere, as an insect species expands its area of distribution from south to north, the northern strains of the species should enter diapause earlier in the season and in greater percentages than those of the south. This is because the winters occur earlier, last longer and generally are colder with an increase in latitude.

B. *Incidence of diapause in field-collected adults fed bolls for 20 days prior to examination.* Because of the great differences in the percentages of diapausing weevils in collections made within a span of a few days, a second method of handling the insects was tested. By this method, adults collected at random in fields near Afton (HP) and College Station (CS) were brought to the College Station laboratory and caged in an open insectary. These adults were fed small cotton bolls for 20 days before being examined. The 20-day feeding period was selected since Brazzel *et al.* (1961) had suggested this is ample time for an adult boll weevil to acquire all the characteristics of diapause, such as the accumulation of fat, atrophy of reproductive organs, and so forth. This technique should have allowed ample time for all adults destined for diapause to attain the state of "firm diapause" described by Brazzel and Newsome (1959) and should minimize the numbers of weevils in "intermediate diapause" as defined by these two authors. Thus, by this technique, differences between diapausing and reproducing individuals should be more clearly defined. The resulting data then should furnish a more accurate estimate of

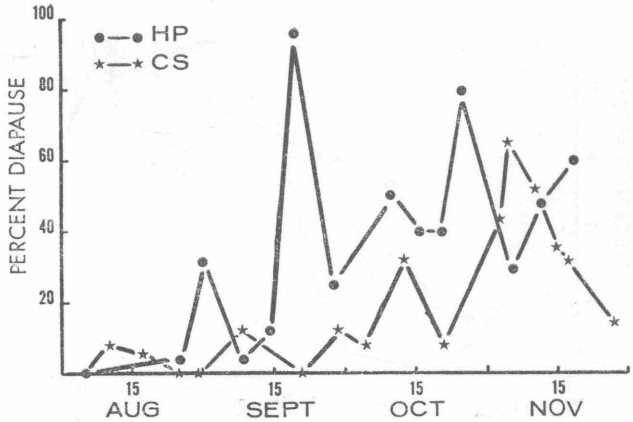

Figure 1. Seasonal incidence of diapause in populations of boll weevils from the High Plains (HP) and College Station (CS). Diapause determinations were made on the date adults were collected in the field. The percentages of diapausing adults were greater on almost every date in the High Plains population than in the one from College Station.

the percentages of potential overwintering weevils in the population than in the case of weevils examined on date of field collection.

Diapause curves made from these data, presented in Figure 2, were considerably smoother than those reported for weevils examined on the date of collection. These data indicate that approximately 20 percent of the adults in the field collections of HP weevils made in August were destined for diapause. Shortly after September 1, the percentages of diapausing adults in the HP population increased steadily to a maximum in November when about 80 percent of the individuals were of the diapause type.

The incidence of diapause in the CS population was small until late September. After this, the percentages of diapausing weevils in this population increased rapidly achieving a maximum of 75 percent in mid-October.

These data show even more clearly the difference in the seasonal incidence of diapause in the two populations. Diapause occurred much earlier in the HP than in the CS population. Also, greater percentages of the HP adults entered diapause.

A comparison of the response curves plotted in Figures 1 and 2 shows that greater percentages of diapausing weevils were detected in adults fed bolls for 20 days prior to examination than in those examined immediately after collection. This method apparently furnished a better estimate of the potential diapausing population because all weevils in the collection that had been "triggered" for diapause had an ample opportunity to achieve a "firm" state of diapause before examinations were made.

C. *The incidence of diapause in square-reared and field collected adults.* Data presented in Figures

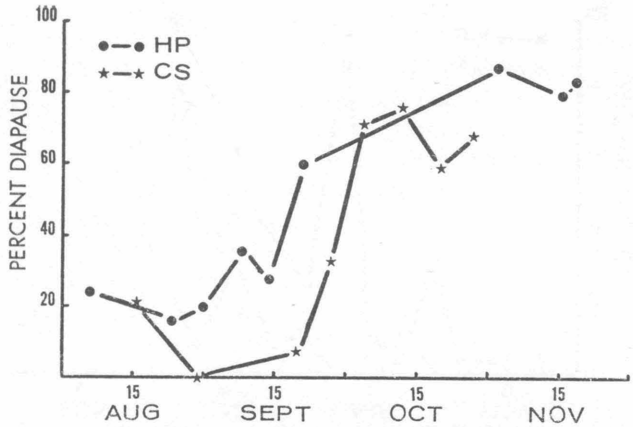

Figure 2. Seasonal incidence of diapause in boll weevils from the High Plains (HP) and College Station (CS) which were fed bolls for 20 days prior to examination for diapause. Dates indicate date adults were collected from fields. The incidence of diapause was much greater in the High Plains population during August and September than in the one from College Station.

1 and 2 show that the percentages of diapausing weevils reported in a collection of field adults on any given date may be influenced to some degree by the method of handling insects after collection. The use of field-collected adults for studying the seasonal incidence of diapause in boll weevil populations may also have other disadvantages. First, the investigator using this method will have little or no information on the age structure of his collection. Thus, the adult collection, particularly those made early in the season, may contain a preponderance of old reproductives formed before the environment demanded any diapause in the population. This would bias the sample in favor of reproductive weevils and would make it difficult to detect the first formed diapausing weevils of the season since their numbers would be small. Secondly, field collections of adults do not take into account any differences in behavior between reproductive and diapausing weevils. Reproductive weevils seek fields having an abundance of uninfested squares or bolls for places to lay their eggs and for feeding. Diapausing weevils apparently do not seek squares or bolls for ovipositional purposes but only for food. Apparently, they do not discriminate as do reproductive weevils between infested and non-infested squares and thus may not be influenced to migrate from heavily infested fields as readily as do the reproductive forms. Because of this difference in behavior, one might logically expect to collect a preponderance of reproductive weevils during late seasons in fields having an abundance of non-infested fruit. Alternately, heavily infested fields with few undamaged squares or bolls may be more heavily invested with weevils destined for diapause simply because the reproductive weevils have migrated to abundantly fruiting fields. Therefore, the incidence of diapausing or reproductive weevils in a sample might be greatly influenced by the fruiting condition of the field chosen for the sample. Results gained from such samples could lead an investigator to erroneous conclusions regarding the seasonal occurrence of diapause in the total boll weevil population. Such samples might also be influenced by the past history of insecticidal treatment of the field. Recently treated fields would have a preponderance of young adults of the same generation while untreated fields might contain adults of one, two or more previous generations. This could greatly affect the percentages of adults in "firm-diapause" in any given sample.

One method for overcoming any difficulty due to sampling would be to collect squares infested with the immature forms of the boll weevil, then hold these in an open insectary under conditions similar to those experienced in the field, and then collect all the adults as they emerge. By this method, one would have weevils of the same age group and would be able to eliminate any bias in the data from behavioral differences between reproducing and diapausing weevils. Also, this method would provide a sample representative of all the weevils being formed in the population during a given span of time.

This procedure was tested during the present study. The adult weevils were reared from infested squares collected on various dates from mid-August to late-November in fields near Afton (HP) and College Station (CS) The squares from both locations were placed in the insectary at College Station and the adults were allowed to emerge. These individuals were fed small bolls for 20 days before being sacrificed for diapause determinations.

Results are presented in Figures 3 and 4. When these data are compared to results in Figure 1 for field-collected adults examined on date of collection, it is obvious that the incidence of diapause in square-reared weevil samples was considerably greater than in samples of randomly collected field adults. These samples show that the incidence of diapause in the HP population in August and in the CS population in early September was much greater than one would expect if only samples of field-collected adults were examined. Data from square-reared weevils appeared to furnish a much better indication of the time of formation of the diapausing individuals than did those collected by the other two methods. These data show that in the High Plains a majority of the adults emerging after September 1, 1964, were of the diapause type. Diapause occurred several days later at College Station, and it was not until late September that more than 50 percent of the weevils emerging at each date entered diapause.

A comparison of square-reared and field-collected boll weevils showed that the incidence of diapause was much greater in the square-reared weevils (Figure 3). This probably was because the infested squares were collected when environmental conditions were appropriate to "trigger" the diapause in the immature stages. The field-collected boll weevils were taken during early fall from fields which had not been treated with insecticides and almost certainly were inhabited with adults of one, two or three previous generations formed at the time when environmental conditions were inappropriate for diapause.

A comparison of the curves plotted in Figure 4 clearly indicates the difference in reaction of the HP and CS populations to the environmental stimuli causing diapause. The onset of diapause occurred about 30 days earlier in the season in the HP population than in the one from College Station. This occurred even though the insects were held in the same location (the College Station insectary) for part of their developmental period and all of their adult life. After October 1, the percentages of diapausing weevils in both populations ranged around 80 percent. These data furnished clear evidence of a difference in the diapause response of the two populations.

The reaction of the boll weevil again shows that as a species spreads from south to north, the northern populations will enter diapause earlier than those from the south. This is as it must be since diapause is a seasonal adaptation by which insects survive the winter. If winter occurs earlier, the insects must enter diapause earlier. Otherwise, they would not be able to survive from one growing season to the next.

Effect of Daylength on Diapause

Earle and Newsom (1964) reported diapause in the boll weevil could be induced by daylengths, or photoperiods, having daily light (L) - dark (D) periods of LD 11:13. By the same token, diapause may be suppressed by LD 13:11. This research led to the present study to determine the photoperiodic responses of boll weevil populations originally collected from near Afton (HP), Presidio (PD) and College Station (CS).

Results, summarized in Figure 5, show that LD 8:16, LD 10:14 and LD 14:10 tended to induce diapause while LD 12:12 and LD 16:8 tended suppress diapause. The incidence of diapause under short days was considerably greater in the HP than in the CS and PD populations. The HP population showed a greater photoperiodic response than the other two populations. This difference in reaction suggests there is a difference in the genetic makeup of the three populations with regard to the photoperiodic response since all were held in identical conditions.

It logically might be suspected that boll weevils in the High Plains should enter diapause earlier in the fall than those at College Station because the growing season in northwest Texas is considerably

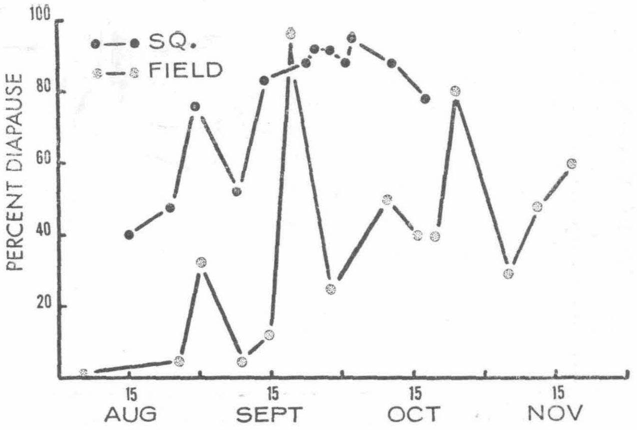

Figure 3. A comparison of the seasonal incidence of diapause in High Plains boll weevils reared from squares in the insectary or collected as adults in the field. Dates are for time of collection of infested squares or field adults.

shorter. The average growing season at College Station is approximately 275 days while that at Crosbyton on the eastern edge of the High Plains averages about 206 days, or 69 days shorter than at College Station. Thus, it should be expected that the boll weevil on the High Plains would have to enter diapause about a month earlier than at College Station in order to avoid death because of an earlier freeze date.

However, environmental differences between the two areas are only part of the story. When weevils from the two locations were reared in identical conditions and exposed to certain photoperiods, it was found that the HP population was significantly more responsive to short days than the CS population. In all diapause-inducing photoperiods tested, greater percentages of the HP population entered diapause than either the CS or PD populations. Thus, it

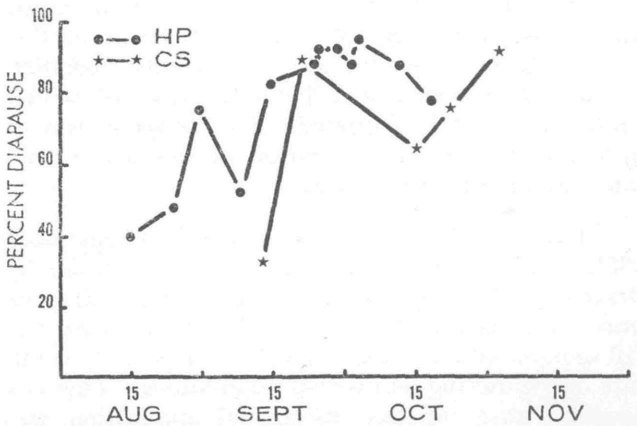

Figure 4. Seasonal incidence of diapause in adult weevils from the High Plains (HP) and College Station (CS) which were reared from squares held in the College Station insectary. Dates indicate the dates on which the squares were collected. The initial onset of diapause was approximately 30 days earlier in the High Plains population than in the one from College Station.

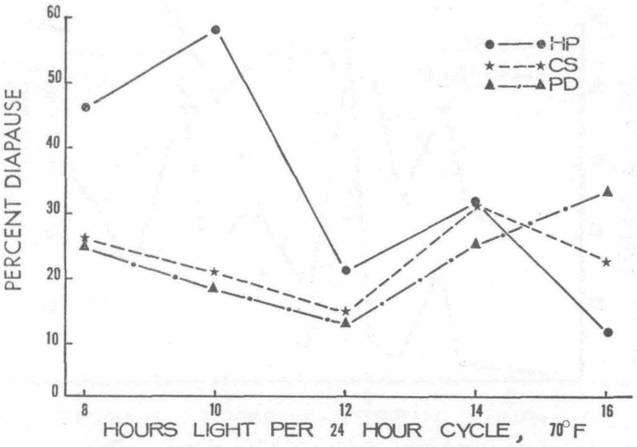

Figure 5. The incidence of diapause in boll weevil populations from the High Plains (HP), College Station (CS), and Presidio (PD) when maintained in photoperiods having from 8 to 16 hours of light per day.

appears that the HP population does possess a different genotype than the other two groups of boll weevils. Apparently the HP population, through the process of natural selection, is becoming adapted to the environmental conditions found on the High and Rolling Plains of Texas. This adaptation has permitted the dispersal and survival of the boll weevil into cotton areas previously uninhabited by the pest.

Importance of the Present Results in Improving the Efficiency of Diapause Boll Weevil Control Programs

A review of the development of the diapause boll weevil control program presently being conducted in the High and Rolling Plains of Texas is a necessary prerequisite to a discussion of methods for improving the efficiency of said program. The first boll weevil invasion of any serious consequence in the High Plains occurred in 1962. First evidence of successful overwintering of the pest in this area was obtained during the winter of 1963-64. As a result of this threat, the producers of the area banded together, raised funds and requested that a diapause boll weevil control program be initiated. This program was to prevent the westward migration of the boll weevil into uninfested cotton areas.

The control program was initiated in September 1964. This program was based on the premise by Brazzel (1961) that four applications of an effective insecticide made at 10 to 14-day intervals during the fall months would materially reduce or possibly eradicate overwintering boll weevil populations. This was provided that the last insecticidal application was timed to occur just prior to frost or to the chemical or mechanical destruction of food and breeding sites of the weevil. Accordingly, a four-application program was initiated on September 16, 1964. It soon became apparent that, because of cultural practices and environmental conditions in the control area, four applications would not extend control until the normal frost date. Therefore, two additional insecticidal applications were made to the most heavily infested part of the control zone. Lack of funds prevented the additional treatment of the entire control zone.

Adkisson *et al.* (1965) evaluated the 1964 program and found that although substantial reductions in overwintering populations had been obtained, a great many weevils survived the applications and successfully overwintered. These populations were of such size that serious crop damage in the control zone during 1965 was prevented only by community-wide control efforts carried out from June to September (Rummel and Adkisson, 1965). These results showed the four- to six-application program aimed soley at killing diapausing boll weevils before they leave cotton fields for overwintering sites was inadequate to obtain the desired population reduction. A more efficient method of control was needed for 1965.

Earlier, Knipling (1963) had advanced the argument that a control program designed to kill the last reproductive generation of boll weevils would be considerably more effective in reducing overwintering populations than a four-application schedule aimed at killing diapausing adults before they leave cotton fields. However, in 1963, knowledge of the seasonal onset of diapause in boll weevil populations was so limited that Knipling's hypothesis could not be adequately tested.

Present results, particularly those presented in Figures 3 and 4 for square-reared weevils, furnish the information needed to implement Knipling's theory. These data show remarkably smooth curves for the seasonal onset of diapause in the boll weevil populations in the High Plains. Under conditions of 1964, the first diapausing boll weevils of the season developed from eggs laid from mid-August to early September. The last reproductive generation, for the most part, occurred in September. The September females produced the majority of the eggs which gave rise to the diapausing, or overwintering, adults. This information provided the basis for revising the diapause control program used in 1965.

The 1965 program was a two-phase program. Phase 1 was aimed at controlling the last reproductive generation of boll weevils by using three applications of insecticide. These applications, made at 5-day intervals beginning September 7, 1965, were designed to kill newly emerging adult females before they could mate and lay eggs. The basic premise behind these applications was to have an "egg-free" period during September when environmental conditions are such as to cause the first boll weevils to diapause.

Phase 2 consisted of four applications of insecticide made at 10- to 14-day intervals beginning at the completion of phase 1. These applications were designed to (1) kill any adult weevils that may have emerged from eggs laid before September 7 and (2) kill any adults that may have survived the September applications. These four applications were scheduled to kill any diapausing weevils that might have developed in the control zone before they could leave the cotton fields for overwintering sites.

The combined effects of the two phased program, as calculated by Knipling (1963), should be as follows provided that the insecticide will kill at least 90 percent of the adults.

1. If 90 percent of the adults of the last reproductive generation are killed, there should be a concomittant decrease in egg production of 90 percent. This means that for each 100 diapausing weevils that would have developed if there had been no control, only 10 will be formed. This provides a 90 percent reduction in the potential overwintering population.

2. If the phase 2 treatments also kill 90 percent of the adults (which by this time will be mostly of the diapausing type), for each 100 potentially overwintering weevils that would have formed if no control had been practiced, only one survives to leave cotton for overwintering sites.

The net effect of the two treatment schedules should be to reduce the population by 99 percent. *More importantly, there should be nine times fewer weevils to survive the new program than the original one.*

This degree of reduction should be particularly devastating to boll weevil populations in the High and Rolling Plains since the winters generally are so severe as to cause additional mortality in the population of 90 percent or more. The combined effect of the diapause treatments and winter mortality should be to reduce the boll weevil population to very small numbers.

LITERATURE CITED

Adkisson, P. L., J. W. Davis, W. L. Owen and D. R. Rummel. 1965. Evaluation of the 1964 diapause boll weevil control program on the High Plains of Texas. Tex. Agric. Expt. Sta. Dept. of Entomol. Tech Rpt. No. 1.

Beckham, C. M. 1963. Seasonal occurrence of hibernation in the boll weevil. Ga. Agr. Exp. Sta. Mimeo. Ser. N. S. 164.

Brazzel, J. R. 1961. Destruction of diapause boll weevils as a means of boll weevil control. Tex. Agric. Expt. Sta. Misc. Publ. 511.

Brazzel, J. R. and L. D. Newsom. 1959. Diapause in *Anthonomus grandis* Boh. J. Econ. Entomol. 50: 603-611.

Brazzel, J. R. and B. G. Hightower. 1960. A seasonal study of diapause, reproductive activity, and seasonal tolerance to insecticides in the boll weevil. J. Econ. Entomol. 53:41-46.

Brazzel, J. R., T. B. Davich and L. D. Harris. 1961. A new approach to boll weevil control. J. Econ. Entomol. 54:723-730.

Earle, N. W., and L. D. Newsom. 1964. Initiation of diapause in the boll weevil. J. Ins. Physiol. 10:131-139.

Knipling, E. F. 1963. An appraisal of the relative merits of insecticidal control directed against reproducing versus diapausing boll weevils in efforts to develop eradication procedures. A letter dated January 28, 1963, addressed to members of the Cotton Insects Research Branch, Ent. Res. Div., Agr. Res. Ser., U. S. Dept. Agric.

Lloyd, E. P., and M. E. Merkyl. 1961. Seasonal occurrence of diapause in the boll weevil in Mississippi. J. Econ. Entomol. 54:1214-1216.

Lloyd, E. P., M. L. Laster and M. E. Merkyl. 1964. A field study of diapause, diapause control, and population dynamics of the boll weevil. J. Econ. Entomol. 57:433-436.

Mitchell, E. R., and W. J. Mistric, Jr. 1965. Seasonal occurrence of diapause and hibernation of the boll weevil in North Carolina. J. Econ. Entomol. 58:309-312.

Rummel, D. R. and P. L. Adkisson. 1965. Unpublished data. Texas A&M Univ., College Station.

Sterling, W. L., S. G. Wellso, P. L. Adkisson and H. W. Dorough. 1965. A cottonseed meal diet for rearing the boll weevil. J. Econ. Entomol. 58:867-869.

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