RESPONSE OF COTTON SQUARES TO VARIOUS BOLL WEEVIL OVIPOSITION PUNCTURE TYPES

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Abstract

In an earlier laboratory study, estimates for boll weevil oviposition in unsealed punctures and punctures sealed with frass (frass-sealed), punctures sealed with a wax film (wax-sealed), and punctures sealed with a wax film plus frass (wax-sealed plus frass) were determined. However, the traditional protuberances of tissue commonly associated with boll weevil oviposition sites were not observed. This study was conducted to determine whether these differing oviposition puncture types would elicit a plant response (i.e., protuberance of tissue at the location of the puncture site) from cotton squares, and, if so, examine the developmental rate of the plant response. All puncture types did elicit a plant response, but the response was not limited to infested punctures (containing eggs and/or larva). Frass-sealed punctures were predominant but other sealed puncture types exhibited comparable infestation rates (82-93%). Despite the high infestation rates in sealed punctures, the overall percentage of infested punctures exhibiting a plant response was only 37.3, 25.7, and 15.3% for frass-sealed, wax-sealed, and wax-sealed plus frass, respectively. Some protuberances were observed at punctures aged ≤24 h, but ≈93% of infested punctures at 24 h had yet to produce a plant response. Punctures that were 48 and 72 h old reflected more protuberances. Data presented here indicates that differing infested puncture types (i.e., wax-sealed, wax-sealed plus frass, and unsealed) do indeed elicit protuberances but require 48 – 72 h to appear in a majority of punctures. These results can supplement the traditional method of detecting oviposition and aid in detection of reproductive boll weevil populations.

Introduction

Accurate and timely detection of reproductive boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), populations in cotton is crucial in areas of the Cotton Belt where this key cotton pest has not been eradicated, and for continued suppression where eradication has been achieved. Boll weevils oviposit primarily in cotton flower buds (squares), and it is commonly accepted that oviposition sites in field situations are characterized by a protuberance (or proliferation of tissue) surrounding punctures sealed with frass.

In addition to punctures sealed with frass, previous reports (Everett and Ray 1962; Greenberg et al. 2003) described other puncture types but failed to provide oviposition estimates of the individual types or simply combined the other puncture types as 'sealed' for purposes of the respective studies. However, in a laboratory study, Esquivel (2007) provided definitive oviposition estimates for these other puncture types that are commonly observed in the field. These other oviposition puncture types were categorized as unsealed, punctures sealed with frass, punctures sealed with a wax film, and punctures sealed with a wax film and partially covered with frass. Because Esquivel (2007) used squares removed from cotton plants and none of the observed punctures produced protuberances, Esquivel (2007) was unable to report whether protuberances were associated with these other oviposition puncture types. Determination of protuberances associated with other oviposition puncture types as indicators of oviposition would further supplement current techniques for monitoring boll weevil populations. Thus, the objectives of this study were to determine whether the unsealed oviposition sites or oviposition sites sealed with frass (frass-sealed), wax film (wax-sealed), or wax film plus frass (wax-sealed plus frass) would cause a plant response (or protuberance) similar to that observed in the field, and to determine the developmental rates of the plant response.

Materials and Methods

Source of boll weevils

Boll weevils were obtained from a concurrent laboratory study, thus, the original source and rearing of known-age boll weevils were identical to that described by Esquivel (2007). Briefly, weevils were reared from field-collected infested squares harvested directly from cotton plants during 2001 and 2002. Infested squares were held at 29.4±1°C, pupae were harvested after approximately 5 d and placed in petri plates containing a moistened layer of vermiculite. Petri plates were checked twice daily for newly eclosed adults, which were sexed using the tergal notch method (Sappington & Spurgeon 2002).

Experimental design

The study was conducted in an insectary under ambient environmental conditions. In this study, 8-d-old mated females from the Esquivel (2007) study were used to determine the temporal response of cotton plants to unsealed punctures and punctures sealed with either frass (frass-sealed), a wax film (wax-sealed), or a wax film plus frass (wax-sealed plus frass), as described by Esquivel (2007). For each insectary trial (4 trials in 2001 and 5 trials in 2002), 15 boll weevil females were distributed among three cages (5 females/cage) housing three greenhouse-reared cotton plants with pristine squares. Each cage represented a puncture age cohort at which affected cotton squares would be removed from the respective plants to assess temporal rate of protuberance development.

For each trial, cotton plants were exposed to boll weevils for 24 h. At the conclusion of 24 h, all plants were removed from respective cages and squares examined for punctures. All punctures were assigned to a puncture category identified by Esquivel (2007) and individually numbered to follow their progress in the development of plant responses to punctures within individual age cohorts. Affected squares were removed from the plants when punctures reached the following cohort ages: ≤24 (hereafter referred to as 24), 48, and 72 h. Cohort ages denote the age at which squares with respective punctures were removed from the cotton plant. For example, the 24 h puncture age cohort denoted squares that were removed from the plant at the conclusion of the 24 h exposure period of plants to boll weevils. For the 48 h puncture age cohort, individual punctures were characterized and assessed for a plant response at the 24 h interval (i.e., the conclusion of the 24 h exposure period) and squares were removed from the plant at 48 h. For the 72 h puncture age cohort, punctures were characterized and assessed for plant response at the 24 h interval (i.e., the conclusion of the 24 h exposure period), re-assessed for plant response at 48 h, and squares removed from the plant at 72 h. Hereafter, these assessments, taken at 24 h intervals will be referred to as puncture age intervals. All removed squares were dissected in the laboratory to assess plant response (i.e., development of tissue protuberance at location of the puncture) in relation to puncture type and boll weevil infestation (presence of egg and/or larvae). Within each trial, cotton plants with pristine, intact squares were provided for three consecutive days (as replicates) in each of the cages.

This report provides preliminary findings of frequencies of observed cotton square responses in relation to types of differing boll weevil oviposition punctures. The data presented here was generated using SAS software, Version 9.2 of the SAS System for Windows XP. The PROC FREQ procedure (SAS Institute 2008) was used for contingency table analyses where presence of a square response were rows and infestation status were columns. This procedure was used for determining overall square response and response of puncture types in relation to infestation.

Results and Discussion

In total, oviposition occurred in 68.3 (n = 1,336), 69.4 (n = 1,166), and 61.7% (n = 1,111) of punctures individually monitored and removed from cotton plants at the 24, 48, and 72 h cohorts, respectively. Because the frequency of oviposition was consistent across the three puncture age cohorts, the number of observations per puncture age interval (i.e., total number of punctures examined at 24, 48, and 72 h intervals) were pooled for analyses. When observations for each age interval were pooled (Fig. 1), only ≈6% of all punctures assessed at the 24 h age interval (n = 3,613) produced a plant response and ≈93% of oviposition punctures (n = 2,407) had not yet produced a plant response. This suggests that a substantial number of oviposition sites could be overlooked because of the absence of a plant response when punctures are ≤24 h old. By the 48 h interval, the percentage of total punctures (n = 2,277) exhibiting a plant response approached 50%, and the frequency of oviposition punctures not producing a plant response was 40.3% (n = 1,494). At the 72 h age interval, a plant response is observed in over half of the observed punctures yet a small percentage of oviposition punctures not producing a plant response (24.1%; n = 685) is still observed.

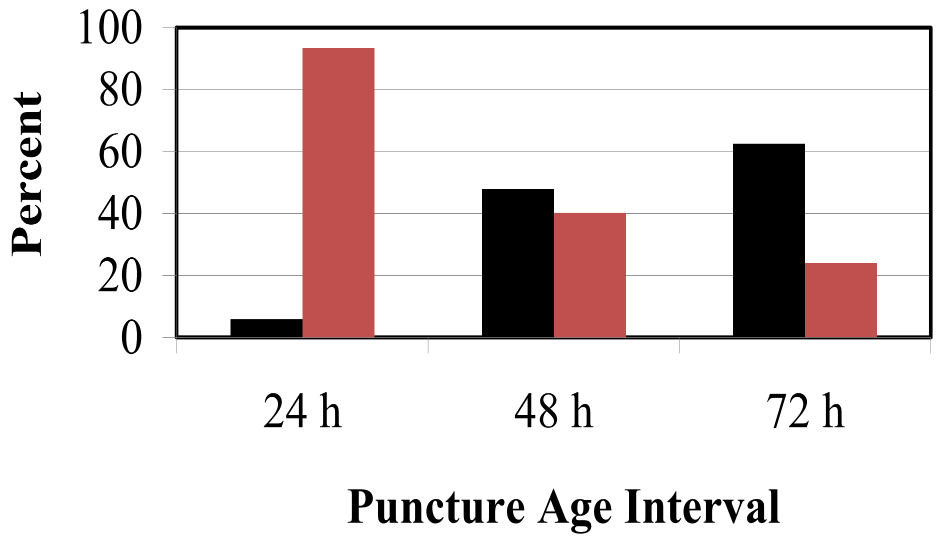

Figure 1. Overall percentage of pooled age interval observations (black bars) where a square response was detected at 24 (n = 3,613), 48 (n = 2,277) and 72 h (n = 1,111) age intervals, and percent of infested punctures (red bars) that did not exhibit a square response at 24 (n = 2,407), 48 (n = 1,494) and 72 h (n = 685) age intervals.

The oviposition puncture types reported by Esquivel (2007) (i.e., frass-sealed, wax-sealed, wax-sealed plus frass, and unsealed; Fig. 2) were all observed in this study. Frass-sealed punctures were predominant, comprising 60.4% of total observed punctures (Table 1); nonetheless, the sealed alternative punctures types exhibited comparable infestation rates (82-93%). The unsealed punctures, commonly accepted to be punctures where feeding occurred, exhibited an 11.9% infestation rate. Despite the high level of infestation rates in punctures with seals, the frequencies of infested punctures with seals that produced a plant response were 37 (frass-sealed), 26 (wax-sealed), and 15% (wax-sealed plus frass). Infested, unsealed punctures also produced a plant response. Thus, all of the puncture types did indeed elicit plant responses.

Figure 2. Representative samples of observed puncture types: left, traditional frass-sealed puncture with protuberance; middle, wax-sealed puncture adjacent to an unsealed puncture; and, right, puncture sealed with wax plus frass.

When the distribution of observed infested punctures at the 24, 48, and 72 h age intervals is examined, Table 1 indicates that infested punctures, regardless of seal type, must be ≥48 h old for ≈50% or more of the punctures to exhibit the traditional protuberance associated with an oviposition site. Twenty-four hours or less are insufficient time for the development of the protuberance in a majority of punctures. Plant responses were not restricted to infested punctures. Responses were also observed for uninfested punctures (4.0 – 43.9%) (Table 1), including those where punctures were unsealed. Even in these uninfested punctures, the trend of time required for the development of a plant response was similar to the trend observed for infested punctures.

Frass-sealed punctures that produce a plant response are the standard criterion used for detecting oviposition by reproducing weevil populations. However, Table 1 indicates that frass-sealed punctures do not always provide a true measure of oviposition, as evidenced by the low frequency of infested frass-sealed punctures exhibiting a plant response and presence of uninfested frass-sealed punctures exhibiting a response. Temporal observations indicated that it is only when infested frass-sealed punctures are 72 h of age that over 75% produce a plant response. However, it should be noted that at 72 h, ≈67% of uninfested frass-sealed punctures also produced a plant response. Therefore, relying solely on frass-sealed punctures with a plant response to detect reproductive boll weevil populations can be problematic in two ways: not all frass-sealed infested punctures produced a plant response; and, the plant response can create false positives as indicated by plant responses associated with uninfested frass-sealed punctures (Table 1), thereby potentially triggering an unnecessary insecticide application.

A potential approach to mitigate the risks associated with solely relying on frass-infested punctures to detect oviposition is to supplement traditional observations with examinations of other punctures with differing seal types. This report demonstrates that puncture age is critical for development of a plant response similar to that observed in the field. Given the similarity in increased frequency of plant responses from 24 – 48 h regardless of puncture type (Table 1), punctures sealed with wax and punctures sealed with wax plus frass could be used to detect oviposition sites. In fact, unsealed punctures should not be completely overlooked as oviposition sites.

Summary

Data presented here clearly indicated that alternative oviposition puncture types described by Esquivel (2007) do trigger a plant response similar to the traditional protuberance on frass-sealed punctures which are used as the standard criterion for detecting oviposition. Further, when using the plant response as a measure of oviposition, improved detection of oviposition is increased when punctures are ≥48 h of age; ≤24 h is insufficient time for a plant response to develop in a majority of punctures. Granted, it is impossible to know the true ages of punctures in a field situation, but these data, at minimum, should make one aware of other potential indicators of oviposition (i.e., punctures with wax and punctures with wax seal plus frass), and the fact that oviposition may have already occurred in observed punctures despite the absence of a plant response. Assessments based on traditional frass-sealed punctures may underestimate the level of weevil reproduction in cotton, and conversely, overestimate fecundity. Thus, the differing oviposition puncture types should be considered as supplementary indicators for accurate and timely detection of reproductive boll weevil populations.

Acknowledgements

This work reports the results of research only. Mention of a proprietary product does not constitute an endorsement for its use by United States Department of Agriculture.

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Table 1. Frequencies of observed punctures and overall plant responses, including the percentage (*n*) of punctures exhibiting a plant response at indicated age for respective puncture type and infestation status.

Puncture sealed with:	*N* (%)[a]	Infested (%; *n*)[b]	Overall percent with plant response[c]	Percent (*n*) of punctures exhibiting plant response[d] at: ≤24 h	48 h	72 h
Frass	4,229 (60.4)	Yes (81.9; 3,464)	37.3	7.4 (1,738)	62.8 (1,170)	77.2 (556)
		No (18.1; 765)	43.9	14.3 (300)	59.5 (259)	67.5 (206)
Wax plus frass	127 (1.8)	Yes (92.9; 118)	15.3	9.5 (105)	66.7 (12)	0.0 (1)
		No (7.1; 9)	11.1	12.5 (8)	0.0 (1)	0.0 (0)
Wax	978 (14.0)	Yes (82.4; 806)	25.7	4.0 (471)	49.4 (235)	72.0 (100)
		No (17.6; 172)	16.3	3.7 (81)	23.3 (60)	35.5 (31)
Unsealed	1,667 (23.8)	Yes (11.9; 198)	26.8	1.1 (93)	42.9 (77)	67.9 (28)
		No (88.1; 1,469)	4.0	0.5 (817)	6.5 (463)	12.7 (189)

[a] *N* = number of punctures per respective seal type; in parentheses: % denotes percent of total observed punctures in the study.
[b] Infested = presence of egg and/or larva(e); in parentheses: % denotes percentage of total number of punctures per seal (*N*) that were infested or uninfested; *n* = resultant number of punctures that were infested or uninfested per respective seal type.
[c] Based on resultant number of punctures that were infested or uninfested, value denotes percentage of respective seal type and infestation status exhibiting a plant response, where a plant response was classified as a protuberance of tissue at the location of the puncture site.
[d] In parentheses, *n* denotes the number of punctures examined at each puncture age.