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# Effect of Seed Texture Layer on Bruchid Infestation in Mungbean *Vigna radiata* (L.) Wilczek

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**ABSTRACT** An experiment was carried out to evaluate the effect of texture layer of mungbean seed on infestation of bruchids (*Callosobruchus maculatus* and *C. chinensis*) (Coleoptera: Bruchidae). Seventeen BC9 near-isogenic mungbean lines (NILs) which are resistant to the insects, and their parents (KPS 1, CN 60 and the wild mungbean TC 1966), were compared. Each entry was prepared in two sets, one with intact texture layer and the other with texture layer removed by 15% NaOH. Each set was divided into 8 samples of 5 g each to test against the bruchids. The results revealed that both bruchid species laid more eggs on no texture layer seeds than on the intact ones. The difference in egg laying was especially high on seed of the wild mungbean TC 1966. The NILs, KPS 1 and CN 60 harbored several eggs on seed surfaces. However, the resistant lines had almost no damaged seed, regardless the removal of texture layer. The highest damage among the resistant NILs was only 5.5% while the susceptible recurrent parents were completely damaged. The texture layer seemed to affect only insect oviposition but not seed damage. Thus, to evaluate for chemical resistance to bruchids in a mixture of dull- and shiny-seeded mungbean, their texture layer should be removed to normalize the number of eggs laid on seeds.

KEYWORDS: *Vigna radiata, Collasobruchus chinensis, C. maculatus*, mungbean, bruchids, seed texture layer.

## INTRODUCTION

Bruchids, *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) (Coleoptera: Bruchidae) are the most devastating and widespread store pests that can infest mungbean in the field as well as during storage. The initial infestation originates in the field, where the adult beetles lay eggs on green pod and the larva bore through the pod and feed in developing seed.[1] When the seeds are harvested and stored, the insects continue to feed, emerge to adults, and cause further infestation which results in total destruction of seed within 3 to 4 months.[2] In storage, the adults lay eggs directly on seed coat. The newly hatched larvae bore through the egg shell and penetrate seed coat to find their food. The female bruchids prefer to lay eggs on smooth surface rather than on rough surface of seeds. The rough (dull) seeds are those covered with inner pod membrane that renders the seed dull. When this membrane is removed, the seed coat underneath is shiny.[3] The pod membrane may contain brown or black pigment through which the seed coat color may not be apparent. Fujii *et al.*[4] observed that seed of the bruchid resistant mungbean TC 1966 is covered with a network of parallel and transverse ridges, compared with smooth surface of commercial mungbean. This characteristic makes the female bean weevil rather hesitant to lay eggs on. Seed size may also play role in oviposition preference. However, the resistant mechanism is reportedly antibiosis type and independent of the physical mechanism.[5] They constituted ar tificial seeds from varying ratios of flour from resistant and susceptible accessions and found that the number of bruchid adults emerged from the artificial seeds decreased with increasing proportion of flour from the resistant accession. Watt *et al.*[3] reported that texture layer can be removed from mungbean seed surface by washing seeds with 15% NaOH solution. Imrie and Lambrides[6] mass screened mungbean germplasm and found that the presence of texture layer and seed size influenced bruchid damages. Seeds with texture layer intact and smaller seeds harbored less eggs of four major bruchid species, *C. maculatus*, *C. chinensis*, *C. phaseoli* and *Acanthoscelides obtectus*. They did not assess further for seed damage. They thus recommended to initially screen large number of accessions using seeds with texture layer intact. Only the resistant accessions will be retested using seeds treated with 15% NaOH to remove the layer. Then resistant accessions from the latter step are identified for further tests or uses.

The current experiment was conducted to identify effect of texture layer on egg laying, number of adults emerged and percent seed damaged from two bruchid species, *C. maculatus* and *C. chinensis*.

## Materials and Methods

This experiment was carried out in the insect laboratory of the Asian Regional Center of the Asian Vegetable Research and Development Center (ARC-AVRDC), Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. Mungbean seed of 17 $BC_9$ near-isogenic lines (NILs) and their parents, 'Kamphaeng Saen 1' (KPS 1), 'Chai Nat 60' (CN 60) and TC 1966 were used to test the effect of texture layer on bruchid infestation. The $BC_9$ NILs were developed by backcross breeding method using the high yielding bruchid susceptible cultivars KPS 1, and CN 60 as recurrent parents and the wild mungbean TC 1966 as the donor of bruchid resistance gene. Backcrossing was performed for nine generations plus one selfing generation to establish each NIL.

**Procedure for removing texture layer**

1. Add 125 to 150 ml of 15% NaOH to a clean 250 ml glass beaker.
2. Add 50 g mungbean seed with a magnetic stirring rod, put on a stirrer to allow stirring at 475-500 rpm for 3 minutes.
3. Pour off the NaOH, then wash seed twice with 125-150 ml distilled water, stirring for 30 sec each time.
4. Pour off water and blot dry the seed immediately with tissue paper.

**Procedure for assessment of bruchid resistance**

Seeds from KPS 1, CN 60 and each NIL were divided into two sets, one with the texture layer, the other with texture layer removed. Each set was subdivided into 8 samples of 5 g each to test against *C. maculatus* and *C. chinensis*. Each sample was put in a 50 $cm^3$ plastic box with 30 adult insects of each species having roughly equally males and females. One week later, the insects were removed from the box and the number of eggs laid on the seed coat were counted. Seeds were kept in the box for 40 days more to allow emergence of new adults. Data were recorded on number of emerged adults, number of good seeds and damaged seeds from which the % damaged seed was calculated.

**Data analysis**

The data on number of eggs laid on 5 g seed were analyzed following the factorial arrangement of treatment combinations in a Completely Randomized Design consisted of three factors, viz. 20 mungbean genotypes x 2 bruchid species x 2 texture layers. Since this experiment has no real replication, the 2- and 3-factor interactions were combined and used as a pooled error to test for significance of the main effects. Data on number of adults emerged and % seed damaged were not normally distributed, however clearly different among the entries, thus they did not require statistical analysis and testing.

## Results and Discussion

The NILs had slightly different weight of 1000 seeds as shown in Table 1. This variation was too small and can be neglected. The number of eggs counted in each seed box revealed that the bruchids laid significantly more eggs on the no texture layer seed than on the texture layer one. The contrast was especially clear in TC 1966. *C. maculatus* and *C. chinensis* laid only 70 and 127 eggs per 5 g intact seeds, while they laid 507 and 311 eggs per 5 g on no texture layer seeds, respectively. Variation in number of eggs laid by different bruchid species and on different seed texture layers were clearly confirmed in the analysis of variance table (Table 2). With the intact-dull TC 1966 seed, the bruchids laid eggs mainly on the plastic box, which is glossier. Although less pronounced, *C. maculatus* females laid more eggs on NIL seeds in the absence of texture layer. The NILs of KPS 1 and CN 60 harbored on the average 344.5 and 365.1 eggs per 5 g seeds as compared to in the presence of texture layer, which harbored 305.3 and 297.1 eggs per 5 g seeds, respectively. Similarly, *C. chinensis* females laid 260 and 272.6 eggs on the no texture layer seeds of NILs of KPS 1 and CN 60, as compared to 166.5 and 184.8 eggs on the texture layer intact seed. Although the NILs had as large and shiny seed as their recurrent parent, eggs laid on the NaOH washed seeds tended to be slightly greater than on the intact ones (Table 1). Almost all eggs were laid on shiny seeds of these lines, only few eggs were found on the plastic box.

Many adults emerged from seed of the two susceptible cultivars, KPS 1 and CN 60 regardless the presence or absence of texture layer, while only few adults were observed from the NIL and TC 1966 seed. Thus there was no relationship between the number of eggs laid and the number of adults emerged. This fact also holds on percent of seed damaged. The damage varied between 0 to 5.5 % in the NILs, whereas 88.9 to 98.7 % were detected in the susceptible cultivars, regardless number of eggs laid. Seed damage percentage was clearly associated with number of adults emerged. Thus it is conclusive that the presence or absence of texture layer on

**Table 1.** Effect of mungbean seed texture layer on reaction to bruchids, *C. maculatus* and *C. chinensis.*

| Entry no. | Cultivars/ lines | 1000-seed weight (g) | *C. maculatus* | | | | | | *C. chinensis* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. eggs laid | | No. adults emerged | | % damage | | No. eggs laid | | No. adults emerged | | % damage | |
| | | | Texture | No texture | Texture | No texture | Texture | No texture | Texture | No texture | Texture | No texture | Texture | No texture |
| 1 | KPS1-2-7-29 | 66.6 | 299 | 378 | 0.0 | 0.3 | 0.0 | 0.3 | 98 | 226 | 0 | 0 | 0 | 0 |
| 2 | KPS1-3-6-37 | 64.0 | 251 | 283 | 0.7 | 0.0 | 0.9 | 0.0 | 135 | 201 | 0 | 0 | 0 | 0 |
| 3 | KPS1-1-4-29 | 67.3 | 322 | 293 | 2.3 | 0.7 | 2.3 | 0.6 | 149 | 287 | 0 | 0 | 0 | 0 |
| 4 | KPS1-4-4-41 | 64.5 | 311 | 277 | 0.5 | 0.0 | 0.6 | 0.0 | 177 | 260 | 0 | 0 | 0 | 0 |
| 5 | KPS1-3-1-35 | 65.7 | 306 | 436 | 0.3 | 0.5 | 0.3 | 0.6 | 251 | 266 | 0 | 0 | 0 | 0 |
| 6 | KPS1-2-9-31 | 66.8 | 238 | 358 | 0.7 | 0.5 | 0.9 | 0.6 | 242 | 284 | 0 | 0 | 0 | 0 |
| 7 | KPS1-3-3-37 | 65.4 | 348 | 399 | 0.7 | 0.5 | 0.9 | 0.6 | 135 | 306 | 0 | 0 | 0 | 0 |
| 8 | KPS1-4-2-39 | 66.4 | 367 | 332 | 0.5 | 0.0 | 0.6 | 0.0 | 145 | 250 | 0 | 0 | 0 | 0 |
| **Average (KPS 1 NILs)** | | **65.8** | **305.3** | **344.5** | **0.7** | **0.3** | **0.8** | **0.3** | **166.5** | **260.0** | **0.0** | **0.0** | **0.0** | 0.0 |
| 9 | CN60-3-1-13 | 69.6 | 274 | 350 | 0.5 | 2.0 | 0.6 | 2.5 | 161 | 291 | 0 | 0 | 0 | 0 |
| 10 | CN60-5-7-24 | 62.2 | 264 | 357 | 3.5 | 2.5 | 5.5 | 3.0 | 203 | 254 | 0.3 | 0.3 | 0.3 | 0.3 |
| 11 | CN60-2-2-11 | 63.8 | 299 | 342 | 3.5 | 0.7 | 3.5 | 0.8 | 145 | 310 | 0.3 | 0 | 0.3 | 0 |
| 12 | CN60-6-7-17 | 74.7 | 289 | 357 | 1.7 | 0.5 | 2.0 | 0.7 | 118 | 245 | 0 | 0 | 0 | 0 |
| 13 | CN60-5-3-20 | 59.6 | 275 | 409 | 0.3 | 2.5 | 0.3 | 2.3 | 205 | 240 | 0 | 0 | 0 | 0 |
| 14 | CN60-6-6-16 | 75.4 | 316 | 289 | 3.3 | 0.3 | 3.7 | 0.3 | 214 | 324 | 0 | 0 | 0 | 0 |
| 15 | CN60-6-3-16 | 79.1 | 314 | 487 | 0.3 | 0.0 | 0.3 | 0.0 | 256 | 265 | 0 | 0 | 0 | 0 |
| 16 | CN60-2-3-12 | 62.8 | 321 | 373 | 1.3 | 3.3 | 1.4 | 2.6 | 225 | 241 | 1 | 1.7 | 0.3 | 0.8 |
| 17 | CN60-2-1-10 | 63.6 | 322 | 322 | 0.3 | 4.5 | 0.3 | 4.9 | 136 | 283 | 0.3 | 0.7 | 0.3 | 0.9 |
| **Average (CN 60 NILs)** | | **67.9** | **297.1** | **365.1** | **1.6** | **1.8** | **2.0** | **1.9** | **184.8** | **272.6** | **0.2** | **0.3** | **0.1** | **0.2** |
| 18 | KPS1 | 68.4 | 178 | 299 | 130.0 | 171.3 | 98.7 | 97.9 | 133 | 260 | 142.5 | 174.5 | 99.7 | 98.1 |
| 19 | CN60 | 69.2 | 292 | 306 | 105.7 | 153.0 | 98.1 | 91.1 | 185 | 286 | 154 | 157.5 | 97.7 | 88.9 |
| 20 | TC 1966 | 16.7 | 70 | 507 | 1.3 | 3.7 | 0.4 | 1.1 | 127 | 311 | 1.3 | 1.3 | 0.1 | 0.3 |
| **Mean** | | | **282.8** | **357.7** | - | - | - | - | **172.0** | **269.5** | - | - | - | - |

**Table 2.** Analysis of variance in number of eggs laid by *C. maculatus* and *C. chinensis* on 5 g mungbean seeds with and without texture layer.[a]

| Sources of variation | Degrees of freedom | Mean squares |
|---|---|---|
| Mungbean lines | 19 | 5,304$^{ns}$ |
| Bruchid species | 1 | 181,832$^{**}$ |
| Seed texture layer | 1 | 163,262$^{**}$ |
| Pooled error[2/] | 58 | 21,113 |

[a] Raw data were obtained from Table 1.
[b] Pooled error is obtained by combining 2- and 3- factor interactions.
ns = non-significant; ** significant at P = 0.01

mungbean seed has nothing to do with seed damaged by *C. maculatus* and *C. chinensis*. The resistance is purely of antibiosis type as reported by AVRDC.[5]

## Conclusion

The presence or absence of texture layer affect oviposition of both bruchids, *Callosobruchus maculatus* and *C. chinensis*, especially in dull texture layer seed of the accession TC 1966. Although texture layer has nothing to do with mungbean antibiosis resistance to the bruchids, it is often necessary that the number of eggs laid on seeds be normalized under a more precise selection process. In this case, the texture layer of the dull-seeded accessions should be removed before use in screening for bruchid resistance to increase the number of eggs laid so that the selection pressure is uniformly imposed on all accessions under selection.

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