EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 4
33-35
Year: 2019

THE ROLE OF SEED COAT THICKNESS IN RESISTANCE/SUSCEPTIBILITY OF LEGUMES TO COWPEA BEETLE (*CALLOSOBRUCHUS MACULATUS*) INFESTATION

Taghread Hudaib*

University of Lincoln, School of Pharmacy, Brayford Pool, Lincoln, LN6 7TS.

***Corresponding Author: Dr. Taghread Hudaib**
University of Lincoln, School of Pharmacy, Brayford Pool, Lincoln, LN6 7TS.

Article Received on 30/01/2019 **Article Revised on 20/02/2019** **Article Accepted on 13/03/2019**

ABSTRACT
The role of seed coat thickness in the resistance/susceptibility of *V. unguiculata* (black-eyed beans, Cultivar: Black-eyed peas) against infestation by the bruchid beetle *C. maculatus* (F.), was investigated. Decoated *V. unguiculata* beans were individually coated in one to four of previously removed seed coats or one layer of *Phaseolus vulgaris* (Red Kidney Beans-British Type) seed coat, before being offered to non-virgin *C. maculatus* females to oviposit on. Following incubation for several weeks, the total adult emergence was then used to determine egg-adult survival and the seed coat thickness for both types of beans was measured. The seed coat of *P. vulgaris* was approximately three times thicker than that of *V. unguiculata*. The survival of larvae in the four coats *V. unguiculata* treatment was similar to that of *P. vulgaris* seed coat, which showed the high correlation of seed coat thickness with *V. unguiculata* beans resistance to *C. maculatus*.

KEYWORDS: Bruchid beetle, Infestation, Seed coat, Thickness.

1. INTRODUCTION

Physical characteristics of seed coat including the thickness are important factors in the resistance or susceptibility of legume seeds to the cowpea beetle *C. maculatus* infestations. Nwanze et al. (1975) examined the oviposition preference of *C. maculatus* across different areas of the host-bean seed-coat and found that textural differences affected the oviposition decisions; females did not lay eggs on the spongy seed hilum. This study concluded that *C. maculatus* prefer to lay eggs on smooth varieties of beans.

Edde and Amatobi (2003) indicate that seed coat thickness has no effect on bean resistance to *C. maculatus* infestation. They came to this conclusion after rearing the cowpea beetles *C. maculatus* on two groups of beans; one group was the normal coated beans and the other with beans that have their seed coat removed. They suggested that the seed coat has no value in cowpea defence against *C. maculatus* attacks. Other researchers have suggested that the role of seed coat thickness in bean resistance is considerable; as example, Kitch et al. (1991) suggested that the seed coat thickness was highly correlated with the resistance of *V. unguiculata* beans to the cowpea beetle *C. maculatus*. Resistance was measured as larval mortality following egg-hatch and before penetration (Pre M) of the seed coat compared to larval mortality after penetrating the seed coat (post M). Thirty varieties of cowpea were examined, ten of them revealed a mortality (from egg hatch to adult emergence) of greater than 95%. The range of mortality within the Pre M larvae was 57.9% to 99.4% and the range within the post M was 6.7%, to 82.6%., which showed the high correlation of seed-coat thickness with *V. unguiculata* resistance to *C. maculatus*.

2. MATERIALS AND METHODS

To explore the effect of seed coat thickness on egg-to-adult survival of *C. maculatus*, *V. unguiculata* beans were soaked in distilled water for 15 minutes and the seed coat removed. Decoated seeds were then individually wrapped in 1 to 4 of the still wet and sticky, previously removed seed coats and then wrapped in aluminium foil. A single 5mm diameter hole was created in the foil to expose a constant surface area of seed coat to ovipositing females. An additional treatment was established by soaking *P. vulgaris* beans that are highly resistant to the cowpea beetle *C. maculatus* (Huesing et al. 1991) in distilled water for 30 to 45 minutes and a single layer of wet seed coat wrapped around the decoated *V. unguiculata* beans, before being wrapped in aluminium foil into which a 5mm diameter hole had been created as previously described by Hudaib et al. (2010). In this experiment the control was the decoated *V. unguiculata* beans wrapped in aluminium foil as described above.

Thirty seeds of each group were placed into separate containers and 4 non-virgin *C. maculatus* females (24 hours from eclosion) added to oviposit for 2h (females

given no choice). Following oviposition the females were removed. The number of eggs laid on seeds was counted and after approximately 4 weeks of incubation at 27oC, 35% r.h., adults started to emerge from the seeds. Total adult emergence was then used to determine egg-adult survival from the six treatments. Each egg was considered a replicate and survival reported as eclosed or not. To determine the thickness of the removed seed coats, small pieces were fixed vertically on a microscope slide by Blue Tack. Seed coat thickness was measured by Motic Images Plus 2. ML software (Fig 2).

3. RESULTS

The number of seed coat layers significantly affected the larval survival (Fig 1); Binary logistic regression showed that treatment affected the likelihood of eggs hatching; change in deviance (Chi-square = 113.5, df = 5 $p<0.0001$). Larval survival decreased as more seed coat layers were added and was also low when seeds were coated in *P. vulgaris* seed-coats. The addition of a single *P. vulgaris* or a single *V. unguiculata* seed coat caused egg-to-adult survival to drop by 82% and 25% respectively, in comparison to the control, decorticated seeds (Fig 1). The addition of further *V. unguiculata* seed coats caused survival to decrease, such that when 4 seed coats were added, egg-to-adult survival had dropped by 83% in comparison to the control.

When the thickness of 10 seed coats of *P. vulgaris* and 10 seed coats of *V. unguiculata* were measured using Motic Images Plus 2. ML software (Fig 2), the seed coat of *P. vulgaris* was approximately three times thicker than that of *V. unguiculata*; the average thickness of *V. unguiculata* seed coat (0.027mm + 0.001) was found to equal approximately 30% of the thickness of the *P. vulgaris* seed-coat (0.088mm + 0.003); (t-test: t = 22.3; df = 18; $p<0.05$).

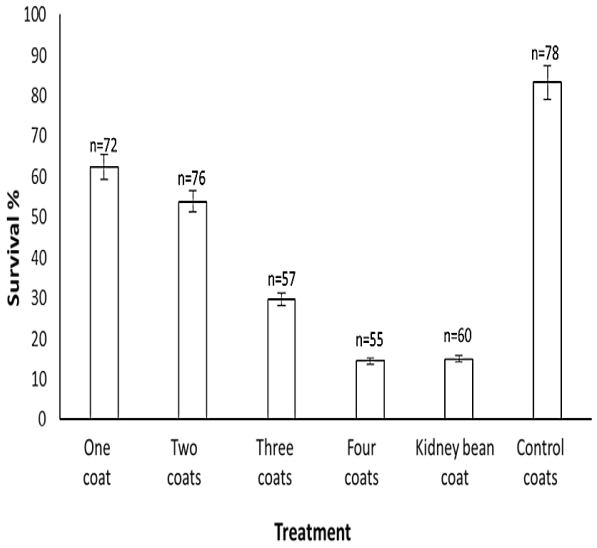
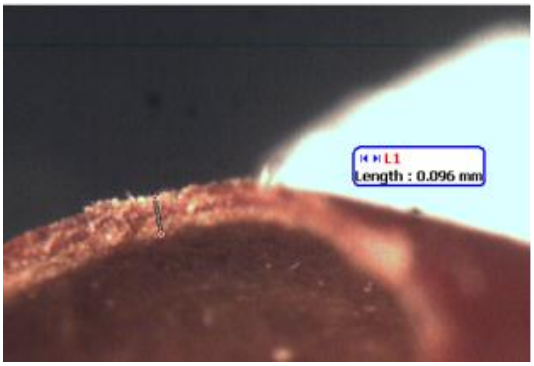


Fig.1: The effect of seed coat layers and species on the survival of *C. maculatus* larvae, n is the number of eggs assayed.



a

b

Fig. 2 a: *P. vulgaris* seed coat section, showing the thickness in mm, b: *V. unguiculata* seed coat section (mm). The thickness is measured with the Motic Images Plus 2. ML software.

4. DISCUSSION

This experiment indicates that *P. vulgaris* and *V. unguiculata* seed coats vary in their effect on the fitness of cowpea beetles *C. maculatus*. The mechanisms of the effects appear to be physical. The ability of larvae to survive to eclosion reduced with increasing seed coat thickness as manipulated by adding additional seed coats to seeds. The survival of larvae in the four coat *V. unguiculata* bean treatment was similar to that of the *P. vulgaris* seed coat treatment, which could be expected on mechanical grounds as the *P. vulgaris* seed coat is approximately three times thicker than the *V. unguiculata* seed coat (Fig 2). These findings are in line with those of Souza et al. (2011), who examined the effect of different seed coat types on larval penetration into artificial seeds along with its effect on larval survival and development. They reported no effect of seed coat type on oviposition but found that seed coat resulted in a reduction of eclosion in some seeds. They also found that the time needed for the first instars to reach the cotyledon varied across the different treatments. Coating artificial seeds with *P. vulgaris* seed coat resulted in penetration of the seed coat by only 55.6% of the first instars. They related this drop to the type of bean that seed coat belongs to and the toxicity of the seed coat but they did not consider the effect of seed

coat thickness. The phytochemical toxicity of *P. vulgaris* seed coat was investigated by Hudaib et al. (2017), they found that phytochemical composition of the seed coat affords some protection against *C. maculatus* by reducing oviposition, disrupting larval growth and reducing survival rates. However, the chemical toxicity did not result in complete survival failure, suggesting that seed coat protection role is derived from other physical or synergistic defence mechanisms. Previous studies report no clear relationship between seed coat thickness and resistance to *C. maculatus* (Janzen 1977; Silva et al. 2004), although Thiéry (1984) did find a direct relationship between seed coat hardness of *P. vulgaris* and the penetration ability of the bruchid *Acanthoscelides obtectus*. Thiéry (1984) also found the seed coats of some resistant legumes to be less hard than those of the *P. vulgaris*.

Here, we highlighted the effect of seed coat thickness as an important factor in legume seed resistance to the cowpea beetle *C. maculatus*. The results suggest the seed coat affords the seed both physical and chemical protection against granivorous beetles. Increasing the thickness of the testa by the experimental supplementation of additional *V. unguiculata* seed coats reduced egg-to-adult survival of *C. maculatus*. This reduction in survival could be a result of a more robust mechanical barrier but it could also result from the increased consumption of metabolites contained within the testa as the first instars pass through the thick seed coat. Thus, the evidence did indicate that the seed coat does play a role in resistance but whether this is due to its physical properties or to its chemical properties remains unclear.

ACKNOWLEDGEMENT

I would like to express my special thanks to my colleague Dr Paul E. Eady for his great help with the statistical analysis used in this article.

REFERENCES

1. Edde P and Amatobi C. Seed coat has no value in protecting cowpea seed against attack by Callosobruchus maculatus (F.). J Stored Prod Res, 2003; 39: 1-10.
2. Hudaib T, Hayes W, Brown S, Eady PE Effect of seed moisture content and d-limonene on oviposition decisions of the seed beetle Callosobruchus maculatus. Entomol Exp Appl, 2010; 137: 120-125.
3. Hudaib T, Hayes W, Brown S, Eady PE Seed coat phytochemistry of both resistant and susceptible seeds affords some protection against the granivorous beetle Callosobruchus maculatus. J Stored Prod Res research, 2017; 74: 27-32.
4. Huesing JE, Shade RE, Chrispeels MJ, Murdock LL α-amylase inhibitor, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. Plant Physiol, 1991; 96: 993-996.
5. Janzen DH How southern cowpea weevil larvae (Bruchidae: Callosobruchus maculatus) die on nonhost seeds. Ecology, 1977; 58: 921-927.
6. Kitch LW, Shade RE, Murdock L L Resistance to the cowpea weevil (Callosobruchus maculatus) larva in pods of cowpea (Vigna unguiculata). Entomol Exp Appl, 1991; 60: 183-192.
7. Nwanze KF, Horber E, Pitts C. Evidence for ovipositional preference of Callosobruchus maculatus for cowpea varieties. Environ Entomol, 1975; 4: 409-412.
8. Silva LB, Sales MP, Oliveira AE, Machado OL, Fernandes KV, Xavier-Filho J. The seed coat of Phaseolus vulgaris interferes with the development of the cowpea weevil [Callosobruchus maculatus (F.)(Coleoptera: Bruchidae)]. An Acad Bras Cienc, 2004; 76: 57-65.
9. Souza AJ, Santos PO, Pinto MS, Wermelinger TT, Ribeiro ES, Souza SC, Deus MF, Souza MC, Xavier-Filho J, Fernandes KV Natural seed coats provide protection against penetration by Callosobruchus maculatus(Coleoptera: Bruchidae) larvae. Crop Prot, 2011; 30: 651-657.
10. Thiéry D. Hardness of some fabaceous seed coats in relation to larval penetration by Acanthoscelides obtectus (Say)(Coleoptera: Bruchidae). J Stored Prod Res., 1984; 20: 177-181.