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Improvement of cowpea grain yields by screening of cowpea lines for resistance to pod-sucking bugs

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Clavigralla tomentosicollis **Stäl, is a notorious pod-sucking bug of cowpea in tropical agro - ecosystems. The improvement of cowpea grain yields by screening of cowpea lines for resistance to pod-sucking bugs has not been undertaken in South Africa. In this study, *C. tomentosicollis* populations were reared on fresh pods of two local cowpea varieties (Glenda and Bechuana White) and one improved variety (IT03K-396-3). Pre-oviposition periods of mated females fed on Glenda, Bechuana White and IT03K-396-3 were 2.5, 7 and 3.8 days, respectively. A longer oviposition period of 56 days was recorded on IT03K – 369 - 3 compared to 51.75 days and 54.75 days on Glenda and Bechuana White, respectively. A significantly (F=8.3, *P* < *0.05*) higher number of eggs were laid by mated females fed on fresh pods of Glenda and Bechuana White than on IT03K-369-3, and lived for a significantly longer period on Glenda (F= 4.4; *P* < 0.05) than IT03K-369-3. Nymphal survival rate was also significantly higher, at 76.7% (F = 9.16, *P* < 0.05) on Bechuana white as well as nymphal weight gain (F = 8.11; *P* < 0.05). Results obtained from this study have significant implications for future research pertaining to cowpea varietal resistance against insect pests in general, and the integrated control of pod-sucking bug infestations on grain legumes in particular.**

Key words: *Clavigralla tomentosicollis*, cowpea varieties, pod-sucking bug, oviposition, development.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp, is claimed to be indigenous to South Africa where several wild relatives exist and it is eaten as dry grains, green pod and tender green leaves (Voster et al. 2007). Cowpea grain and leaves have a high protein content (about 25%), and are rich in other essential nutrients such as calcium, iron, thiamine and riboflavin (Bressani, 1985; Quass, 1995; Voster et al., 2007). Cowpea is an important source of dietary protein in the tropics and subtropics, where consumption rate of animal protein is very low (Voster et al., 2007). Some local cultivars of cowpea cultivated in South Africa include Rhino, Glenda, Chappy, Encore, Rusty, Agri-nawa and Bechuana White. However, a major constraint to sustainable production of this legume is is insect pests. Major cowpea field pests in South Africa, include aphids, lepidopteran insects, and pod-sucking pods, with their importance varying with locality. A complex of pod-sucking bugs attack the pods and seeds of cowpea, of which *Clavigralla tomentosicollis* Stäl is the dominant species (The Mcknight Foundation CCRP, 2006). *C. tomentosicollis* sucks the sap from cowpea pods, causing them to shrivel and dry prematurely, with resultant losses in yield estimated between 20 -100% in various parts of Africa (Singh and Allen, 1980).

The use of chemical insecticides, in practice appears to be the major control measure to pod-sucking bugs (Singh and Jackai, 1985; Jackai and Adalla, 1997). Chemical insec-ticides however, have detrimental effects to humans and the environment, and also are not affordable to a majority of peasant farmers (Alebeck, 1996). The development and use of resistance legume varieties offer a simple, cheap and attractive approach to the reduction of pest damage (Ofuya, 2001). The potential of using plant

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resistance in cowpea for the control of pests has received considerable attention (Singh et al., 1990; Tanzubil et al., 2008).

Crop varieties differ in their phenology and susceptibility to different pests, and it has become difficult to find a single variety that meets subsistence and commercial farmer requirements, such as high yield potential, resistance to field insect pests and diseases, good processing and cooking qualities, palatability as well as optimal storage traits (Crompton et al., 1993). Host plant resistance could be manifested in several ways including, increased mortality of juveniles and /or adults stages of potential insect pests as well as prolongation of the development period of these pests due to antibiotic effects of chemicals present in the host plant. While integrated pest control approaches that employ host plant resistance have been carried out for grain legumes (ground nut and soybeans) in South Africa, no such work has been conducted on cowpea to sustainably control its insect pest complex. Furthermore, research designed to identify cowpea tolerant lines could be used in hybridization programmes for the development of genotypes that are resistant to these insect pest. This study therefore seeks to investigate the relative susceptibility of selected local and improved varieties of cowpea to various developmental stages of *C. tomentosicollis*.

MATERIALS AND METHODS

Insect culture

Adults of *C. tomentosicollis* used to establish the cultures were collected from a cowpea farm located on the campus of the Walter Sisulu University, Mthatha, Eastern Cape Province, South Africa. The bugs were reared on uninfested fresh pods of Glenda, Bechuana White and IT03K-369-3 cowpea varieties inside transparent plastic cages measuring 15.0 x 10.0 x 7.3 cm in the Zoology laboratory of the Department of Biological Sciences, Walter Sisulu University, South Africa.

Holes were made on each side of each plastic cage and covered with fine nylon mesh for aeration, while a plastic vial filled with water and plugged absorbent cotton wool (for water supply to the insects) was inserted through the fourth hole. The cotton wool was changed every other day. The insects were reared under ambient temperature (25ºC ± 3.00) and relative humidity (34.1 - 75.0) in the laboratory. Bugs were then maintained on fresh green cowpea pods harvested from the farm. In order to maintain a viable culture of the insects, the culture was renewed regularly with fresh population of insects from field collections and eggs that were laid by females were allowed to develop inside the cages and at maturity, excess adults were taken out of the cages and put into other cages to mature and subsequently lay eggs.

Oviposition and longevity

A pair of teneral adults was placed in 10 replications in sealed plastic cages with fresh green cowpea pods of Glenda, Bechuana White and IT03K - 369 - 3. The experiment was laid out in RCB design. Daily observations were recorded on mating, oviposition period and fecundity of mated females. The insects that died were replaced with teneral adults of the same sex and age in order to ensure pairing for mated sexes. Egg batches laid were collected daily from the cage and incubated on moist filter paper inside petri-dishes. Observations were also recorded on the longevity of the 10 mated females reared from the different varieties.

Nymphal growth and development

Fifteen neonate first instar nymphs were reared on fresh maturing pods from Glenda, Bechuana White, and IT03K-369-3 cowpea varieties, respectively in four replications. The experiment was laid in RCB design. Daily records were taken on moulting, nymphal weights and survival as well as developmental period until the adults emerged. The cages were regularly cleaned and replenished with pods from the three varieties.

Statistical analysis

Data from the experiment were subjected to analysis of variance (ANOVA) using SPSS version 17 for windows and means were separated by the least significant difference (LSD) technique at the 5% level of probability.

RESULTS

Oviposition and longevity

The pre-oviposition periods of the mated females in the laboratory were 2.5, 7.0 and 3.8 days for the insects reared on Glenda, Bechuana White and IT03K-369-3, cowpea varieties respectively (Table 1). A longer oviposition period of 56 days was recorded on IT03K-369-3 compared to a shorter period of 51.8 days and 54.8 days on Glenda and Bechuana White, respectively (Table 1). Also, a relatively higher daily rate of oviposition of 12.2 eggs /day was recorded on Glenda, and this was not significantly different from that on Bechuana White (11.4 eggs /day) and on IT03K-369-3 (10.2 eggs /day) (Table 1). Mated females lived significantly longer on Glenda ($F = 4.4$; $P < 0.05$), compared to IT03K - 369 - 3. The bugs laid more eggs on Glenda and Bechuana White com-pared to IT03K-369-3, with the highest number of eggs laid within the 3rd and 8th day of oviposition. The number of the eggs deposited by the females decreased as the females advanced in age (Figure 1). A significantly higher number of eggs, were laid by mated females that fed on fresh pods of Glenda and Bechuana White (140 and 134, respectively) compared to 97.5 eggs on IT03K-369-3 ($F = 8.3$, $P < 0.05$) (Table 1).

Nymphal growth and development

The average egg incubation period ranged from 8 to 9.5 days (Table 2), and the emerging neonate nymphs were successfully reared through 5 instars, emerging as adults, on all of the three cowpea varieties. The mean developmental period (nymph to adult) was longer on IT03K-369-3 (21.8 days), though not significantly different

Table 1. Oviposition and longevity of *Clavigralla tomentosicollis* reared on fresh pods of different cowpea cultivars (range in parenthesis).

Cowpea varieties	Pre-oviposition (days)	Oviposition (days)	Fecundity (eggs)	Oviposition rate (eggs / day)	Longevity of mated females (days)
Glenda	2.8 ± 0.5a (2- 4)	51.8 ± 4.5a (52- 62)	140 ± 59.5a (51-315)	12.2 ± 1.2a (11-16)	109.7 ± 7.9a (80-130)
Bechuana white	7 ± 1.7a (2-10)	54.8 ± 2.6a (51- 62)	134 ± 40.1a (50-243)	11.4± 2.9a (8-20)	97.3 ± 8.1ab (84-140)
IT03K-369-3	3.8 ± 1.4a (2- 8)	56 ± 6.4a (45- 68)	97.5 ± 29b (51-172)	10.23± 0.9a (9-13)	85 ± 7.7b (72-130)
LSD (5%)	4.2	15.2	30.9	6.1	20.5

Fecundity of mated females significant at F = 8.3; P < 0.05; Longevity of mated females significant at F = 4.4; P < 0.05. Mean values (± SE) in each column followed by the same letter are not significantly different at α = 0.05 level (ANOVA followed by LSD test).

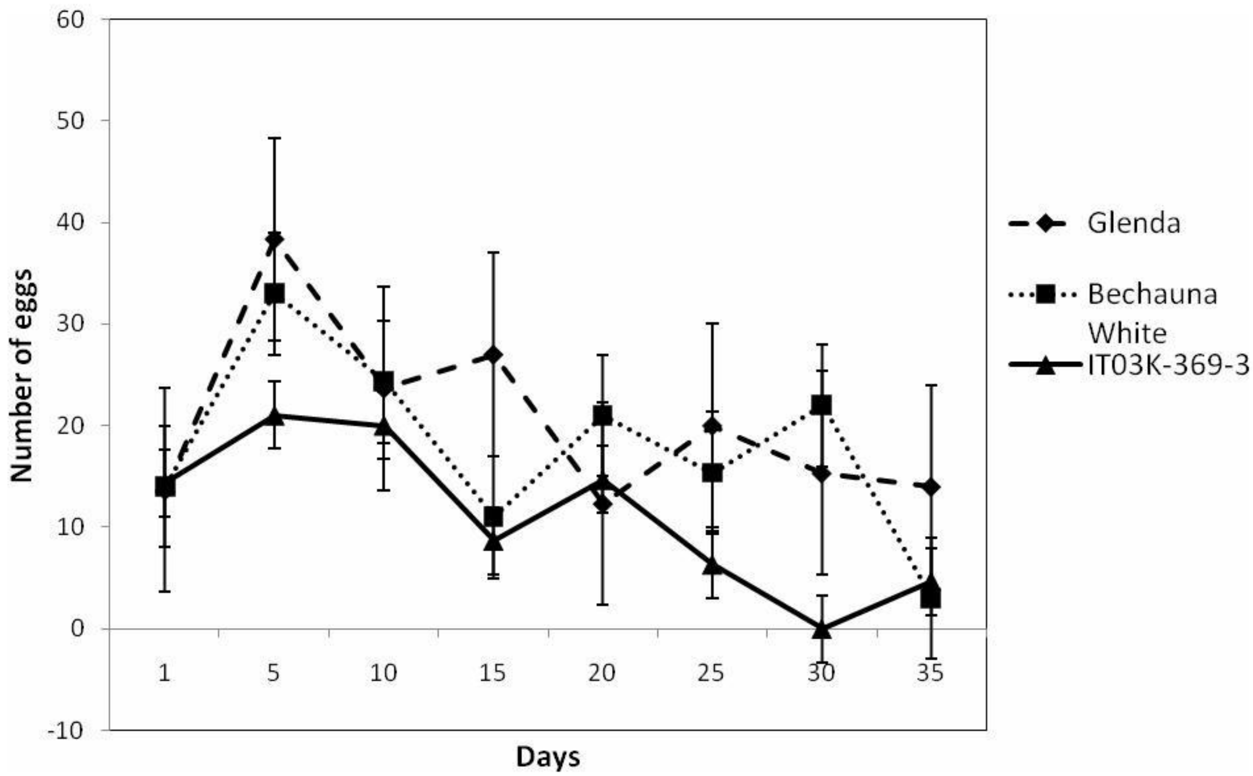

Figure 1. Fecundity of *C. tomentosicollis* on Glenda, Bechuana White and IT03K-396-3 during a five week period of oviposition.

from that of nymphs fed on Glenda (20.5days) and Bechuana White (20.5days) (Table 2). The duration of nymphal development from the first to fifth instar, ranged from 3 to 4 days, with penultimate instars taking 6 to 8 days to moult into young adults (Table 2). Nymphal survival rate was significantly higher, 76.7% (F = 9.16, P<0.05) on Bechuana White than on IT03K-369-3, 59.9% and Glenda, 53.3% (Table 3), with survival percentages lowest in the second instar nymphs. Average nymphal weight gain of *C. tomentosicollis* reared on the three cowpea varieties was significantly higher (F = 8.11; P<0.05) on Bechuana White than on Glenda and IT03K-369-3 (Table 4). The nymphal weight gains were relatively lower in the first, second and third instar nymphs, as compared to the fourth and fifth instar nymphs (Figure 2).

DISCUSSION

Host plant preferences by pod-sucking bugs could result in various cowpea varieties having different susceptibility levels to these pests. This interaction may further influence the life history characteristics of potential insect pests associated with the crop such as pre-mating, mating, oviposition, nymphal development and longevity. The significantly higher fecundity, daily rate of oviposition, and longevity of mated females of *C. tomentosicollis*

Table 2. Duration of egg incubation and nymphal development (days) of *C. tomentosicollis* reared on fresh pods of different cowpea cultivars (range in parenthesis).

Cowpea varieties	Egg incubation	Duration(days) of development nymphal instars					Mean developmental period
		1st	2nd	3rd	4th	5th	
Glenda	8.5 (7-10)	3.75 (3- 4)	3.25 (3- 4)	3.5 (3- 4)	4.0 (3- 5)	7.0 (6- 8)	20.5 ± 0.3a (21-22)
Bechuana white	9.5 (7-12)	4.0	3.0	3.25 (3- 4)	4.0 (3- 5)	6.25 (5- 7)	20.5 ± 0.7a (19-22)
IT03K-369-3	8 (7-9)	4.0	3.0	3.5 (3- 4)	4.0 (3- 5)	7.25 (7- 8)	21.8 ± 0.5a (21- 23)
LSD (5%)							1.58
CV (%)							4.64

Mean values (± SE) in each column followed by the same letter are not significantly different at α=0.05 level (ANOVA followed by LSD test) .

Table 3. Nymphal survival (%) of *C. tomentosicollis* reared on fresh pods of different cowpea cultivars.

Cowpea varieties	Nymphal instars					
	1st	2nd	3rd	4th	5th	Total nymphal survival (%)
Glenda	85.0	80.5	88.2	97.9	92.3	53.3 ± 2.7b
Bechuana White	95.0	90.9	94.8	98.1	96.2	76.7 ± 4.3a
IT03K-369-3	85.0	87.0	91.4	97.5	91.7	59.9 ± 6.7b
LSD (5%)						15.49
CV (%)						15.3

Percentage nymphal survival significant at F=9.16; P<*0.05*. Mean values (± SE) in each column followed by the same letter are not significantly different at α=0.05 level (ANOVA followed by LSD test).

reared on Glenda and Bechuana White are indications that the bug was more adapted to these local cowpea varieties as a source of food than the improved variety (IT03K-369-3). The fewer number of eggs laid and shorter lifespan of the bugs reared on IT03K-369-3, clearly indicated a degree of host resistance and lower susceptibility of this variety to *C. tomentosicollis*. This also points to the presence of antifeedants that may have accounted for the reduced growth of the bug on this improved variety. Similar results were obtained in a report by Koona (1999). A mated female of *C. tomentosicollis* has the potential to lay an average of about 200 eggs in its life time (Singh and Jackai, 1985). In this study, the average number of eggs laid was lower (140), possibly due to sub-optimal rearing conditions in the laboratory as compared to natural environmental conditions characteristic of sub-tropical climates.

The nymphal development of *C. tomentosicollis,* was successfully completed on fresh pods of the three cow-pea varieties, indicating that these varieties are important hosts for the bug. The highest nymphal developmental period was 21.8 days. Similar studies in the tropical west African country of Nigeria showed that the nymphs of this bug took 17.2 days to reach young adult stages (Jackai et al. 1989). The difference in this case was probably due to the effects of sub-tropical climatic variables as the synergistic effects of tropical temperatures and humidity can have profound effects on insect growth and development (Dabire et al., 2005). Different legume food sources have been shown to significantly affect the duration of nymphal development in *C. tomentosicollis* (Egwuatu and Taylor, 1976). In this study, nymphs developed faster when reared on fresh pods of Bechuana White, even though this was not significantly different from that obtained on Glenda and IT03K-369-3. Slansky and Panizzi (1987) have reported that differences in the suitability of the pods and seeds of different plant species to support growth, development and reproduction of seed-sucking insects could be associated with differences in nutrients, allelo-chemicals and physical factors. The presence of various allelo-chemicals such as alkaloids, saponins and protease inhibitors in Leguminosease has also been reported by Weder (1981). These chemicals generally determine the levels of resistance or susceptibility of crop varieties to insect pests, and may have accounted for the lower susceptibility of IT03K-369-3 to *C. tomentosicollis* in this study.

Significantly higher nymphal survival and weight gain on Bechuana White recorded in this study, indicates that this variety is the most suitable for nymphal development of the three cowpea food sources. More bug population infestation will therefore establish on this variety than the others. Growing such a variety on a large scale will lead to a higher yield loss or damage by *C. tomoentosicollis,* if no other cowpea pest control interventions are implemented. The fact that the fourth and fifth instar nymphs of

Table 4. Nymphal weight gain (g) of *C. tomentosicollis* reared on fresh pods of different cowpea cultivars.

Cowpea varieties	Weight gain (g) of nymphal instars					Mean weight change
	1st	2nd	3rd	4th	5th	
Glenda	0.00049	0.00157	0.00481	0.01679	0.02001	0.04365 ± 0.001ab
Bechuana white	0.00039	0.00143	0.00433	0.01826	0.02428	0.04870 ± 0.002a
IT03K-369-3	0.00029	0.00156	0.00321	0.01362	0.02160	0.04119 ± 0.001b
LSD (5%)						0.00604
CV (%)						21.4

Mean weight gain significant at F= 8.11; P<*0.05*. Mean values (± SE) in each column followed by the same letter are not significantly different at α = 0.05 level (ANOVA followed by LSD test).

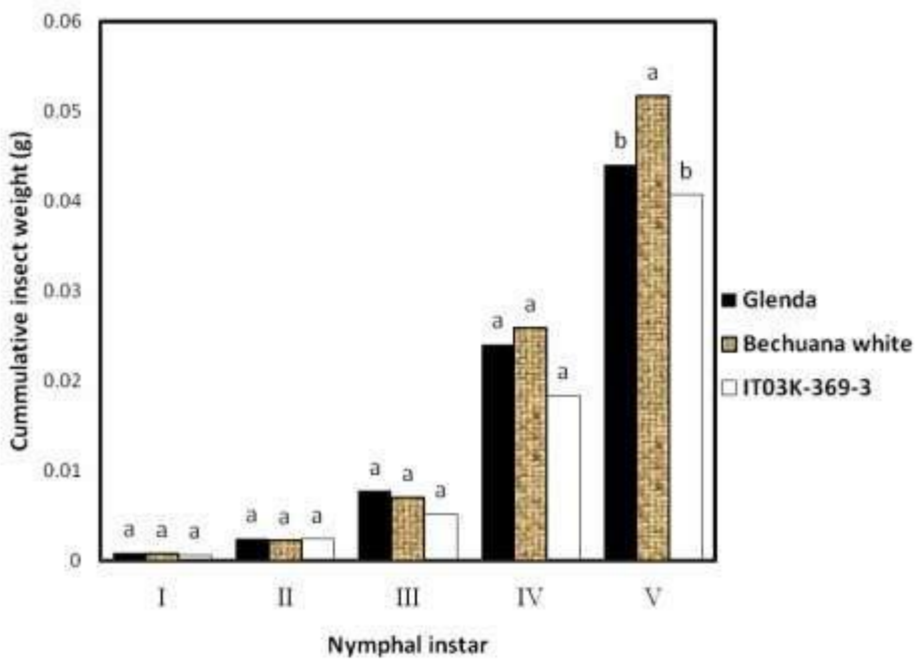

Figure 2. Weight of *C. tomentosicollis* nymphs on fresh pods of different cowpea varieties. Bars with the same letter in each nymphal development stage are not different at 5% level of significant using LSD test.

C. tomentosicollis recorded a relatively higher weight gain compared to the first, second and third stages relates to the ability of these nymphs to inflict greater pod damages that can result in greater yield losses than the lower nymphal and adult stages. This trend was also observed by Soyelu and Akingbohungbe (2006). The ultimate and penultimate instar nymphs of different species of PSBs have been shown to have higher concentrations of digestive enzymes (proteinases) compared to the other instar nymphs and adult stages (Soyelu et al., 2007). Penultimate instars need higher nutrient intake to support their larger body sizes as well as provide sufficient energy that is needed for the last moult into young adult bugs. In this preliminary study, significantly lower numbers of *C. tomentosicollis* eggs deposited, shorter lifespan of mated females and lower nymphal weight gain were observed with IT03K-369-3 cowpea variety. The results imply no final conclusion about the resistance of this particular variety to the pod-sucking bug growth and development but indicate the need for further studies to identify cowpea lines which show resistant traits that could be use in breeding programmes to develop host plant resistant genotypes.

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