Influence of secondary host plants on the embryonic and larval development of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae, Bruchinae).

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Abstract— *The aim of this research was to investigate the influence of secondary host plants on the embryonic and larval development of C.maculatus. The influences of three secondary host plants were compared to the influence of cowpea (Vigna unguiculata (L.) Walp.), the primary host plant in the life cycle of C. maculatus. For the experiments, C.maculatus adults were extracted from cowpea seeds and transferred to the seeds of the two secondary host plants used in this study: striped or white Bambara groundnut (Vigna subterranea (L.) Verdc.) and pigeon pea (Cajanus cajan (L.) Millsp.). Transferred insects were maintained for two years on the seeds of these plants by regular rearings before being used in each of the different experiments. To determine embryonic development time, couples were brought into contact with different seeds for laying. The eggs that were laid were then observed until they hatched. To identify the different larval stages and determine their respective development time, the seeds were first infested and then dissected at specific intervals in time. The results show a reduction in the embryonic development time in white Bambara groundnut seeds compared to the seeds of the other plants. Development time of the two early instars for all strains was significantly long on the three secondary host plant seeds than on cowpea seeds. However the opposite trend was observed for the pupalstage. Of the three plants used in this experiment, pigeon pea provides the worst conditions for larval and pupalinstar development. These results lead to better understanding of post-embryonic development of C. maculates occurring within the seeds of secondary host plants. This research provides valuable insight into developing appropriate methods for pest control.*

Keywords— pest management, secondary host plants, C. maculatus, embryonic and larval development, adaptation, primary host plant.

I. INTRODUCTION

Insects in general and beetles in particular, are endowed with a behavioral plasticity which allows them to adapt readily to plants, whether they belong to the same family or not[1;2;3]. The diet of insects of the subfamily Bruchinae, to which *Callosobruchus maculatus* (Coleoptera: Chrysomelidae, Bruchinae) belongs, is characterized by a high degree of host specialization; their larvae are found inside the seeds of a small number of host plants species[4;5]. *Callosobruchus maculatus* is a cosmopolitan pest species of cowpea in the tropics and subtropics of the world and an important field-to-store pest of pulse crops in Africa and Asia[6]. Females lay eggs on the pods or seeds. After hatching, the larva crosses the tegument and penetrates into the seed. Larval development occurs inside the seed at the expense of food reserves located in the cotyledons and the germ [7]. Grain legumes, especially cowpea (*Vigna unguiculata* (L.)Walp.) and Bambara groundnut(*Vigna subterranea* (L.) Verdc.),are very important in the diets of rural communities as rich sources of protein [8;9]in West Africa. Pigeon pea (*Cajanus cajan* (L.) Millsp.)has also become a very important grain legume used in human nutrition in this area. However, because of their high susceptibility to several storage pests, they are out of reach during a long period of the year, and their nutritive potential is therefore underutilized [10;3]. Many studies have shown that cowpea is the favored host plant for development of *C. maculatus*. However, it is possible that this pest could also develop on unusual host species such as Bambara groundnut and pigeon pea [11;3].Some plants that are known to be favorable to *C. maculatus* allow its development with varying degrees of success [12;3]. Therefore, it is necessary to examine whether *C. maculatus*, which is already a serious pest of cowpea, could also become a key pest of these legumes. Within the Papilionoideae, most hosts of *C. maculates* belong to the tribe of Phaseoleae and crops which are most severely infested, are found in the genus *Vigna*[11].[7]have shown that *C. maculatus* had a longer post-embryonic development on *Flemingo congesta* seeds than on cowpea. Within the genus *Vigna*, some cowpea varieties are more susceptible than others and therefore are more readily infested [12;13].

While this beetle is oligophagous, its development capacity in the seeds of legumes such as *Vicia faba* (L.) remains limited [1]. These plant species seem to contain substances that would block larval development of *C. maculatus* within the seed.

These substances have an antibiosis effect on the growth of *C. maculatus*. They may also influence the speed of *C. maculatus* embryonic development. Understanding the conditions of developing a pest on a specific host is not only important for pest control, but also for the prediction and prevention of the emergence of new pests. *Callosobruchus maculatus* life cycle has four larval stages. The reproductive capacity and development of *C. maculates* have been the focus of much research, the results of which appear to depend on the geographical origin of the strains, the legume species, and cultivar of each host species [14;15]. However, data on the embryonic and larval development of *C. maculatus* are very scarce. Thus, the understanding of embryonic and post-embryonic development and the factors influencing them is a challenge that needs to be addressed for successful integrated pest management. This study is based on the premise that the secondary host plants could influence the larval and embryonic development of *C. maculatus*. The aim of this work was to investigate the influence of two secondary host plants (white and striped Bambara groundnut and pigeon pea) on the embryonic and larval development of *C. maculatus*.

II. MATERIALS AND METHODS

2.1 Insect Origin and mass rearing

The strain of *C. maculatus* used for the experiments was isolated and maintained for one year on the seeds of cowpea. Mature couples were then isolated, transferred and maintained on seeds of two varieties of Bambara groundnut (white and striped) and pigeon peas for two years by regular rearing. This process resulted in the production of three strains of *C. maculatus*: B, C and D respectively. The control (A) was raised on cowpea seeds.

The mass rearing method used has been described by [16]. Adults of *C. maculatus* used, belong to the "non-sailer" form. Newly emerged couples were introduced into Plexiglas boxes (18 x 11 x 04 cm), containing healthy seeds of each legume. Adults were removed after 48 hours of contact with the seeds. Infested seeds were placed in a growth chamber with the standard rearing conditions (T = 32 ° ± 0,1 ° C, RH = 36 ± 1%) and followed until adult emergence. The new generation was reused to maintain strains.

2.2 Origin of secondary host plants

Pods of cowpea (*Vigna unguiculata*), Bambara groundnut (*Vigna subterranea*) and pigeon pea (*Cajanus cajan*) were harvested at Kamboinsé (area of Ouagadougou in Burkina Faso) and shelled. The seeds were sieved and sorted to eliminate those carrying eggs or containing nymphs of beetles or those that were perforated. The rest were placed in a freezer at a temperature of -18°C for one (1) week to eliminate any remaining infestation before the experiments. Physical characteristics such as the diameter and roughness of the seeds were described by [17].Chemical and mineral composition of Bambara groundnut seeds were studied by [18] and [19] respectively.

2.3 Influence of secondary host plants on *C. maculates* embryonic development time.

The experiments were performed with insects growing on cowpea seeds compared to insects growing on secondary host plant seeds.

In the first part of the experiment, pairs of naive *C. maculatus* (1-2 days old) from cowpea were grouped into four batches: A, B, C and D. These insects were placed in contact with cowpea, Bambara groundnut (white and striped) and pigeon pea seeds respectively. Next, we introduced two pairs of *C. maculates* to a Petri dish of 20 g of seeds from one of the four host plants. After 24hours of contact, insects were removed and infested seeds were maintained under the standard rearing conditions (T = 32 ± 0.1 ° C and RH = 36 ± 1%). For each seed type, the same operation was repeated four times.

Next, 200 seeds were collected from each batch of infested seed, each with one fresh egg. This was transferred to new Petri dishes where daily observations of the eggs were made through binocular loupes to note hatching. Newly hatched eggs were characterized by a black spot. This spot was the head capsule of the larva coming from the newly hatched egg. Whenever an egg hatched, we removed the egg-bearing seed from the batch. Hatching dates were noted from the beginning to the end of the experiment. This procedure permitted the estimation of the embryonic development time of *C. maculates* for these legume species.

In the second part of the experiment, pairs of naive *C. maculatus* (1-2 days old) were again grouped into the four batches: A, B, C and D from cowpea, white and striped Bambara ground nut and pigeon pea respectively. These insects were reared and maintained by regular rearings on these seeds for two years before the beginning of the experiment. Next, the same procedure as the first part was maintained.

2.4 Influence of secondary host plants on the development time of immature stages of *C. maculatus*.

The experiment was carried out after insects were reared and kept on the seeds of each secondary host plant for two years. We provided four batches of seeds for the control strain and four for each of the secondary host plants. Each batch consisted of four plexiglass boxes each containing 250g of healthy seeds from one of the used plants. In each box, we introduced 20 couples of *C. maculatus* (1-2days old). After 24 hours of contact, the insects were removed and infested seeds were placed in rearing conditions (T = 32 ° ± 0,1 ° C, RH = 36 ± 1%) and observed.

Every two days from the 6th day after infestation to the emergence of the first adult, 20 seeds of each plant were removed and placed in a freezer to stop larval development. After 72 hours, the seeds were removed and immersed in water for ten hours. Then we proceeded to the dissection of these seeds in order to collect larvae. Fifty larvae per seed type and date of removal were collected, classified according to their age, and stored in alcohol at 70°C. We subsequently carried out the body measurements of larvae from different batches using a verniercaliper. Then, these larvae were described based on morphological criteria such as the size, general appearance, the presence or the absence of the head capsule (the first stage) and posture [20; 21]. We determined the stage of larvae for those where their age was already known. Lastly, we estimated the average time of development of the larval stages (expressed in days) from their age.

2.5 Statistical analysis of data

Data were analyzed by using Multivariate analysis of variance (MANOVA)with SAS version 8 [22]. When the overall effect of the host plant taxa was significant, we examined differences between the host plant taxa using Fisher's LSD at the significance level of 5%.

III. RESULTS

3.1 Embryonic development time

The values obtained on the embryonic development time of *C. maculatus* on the seeds of the secondary host plants are presented in Figure 1.

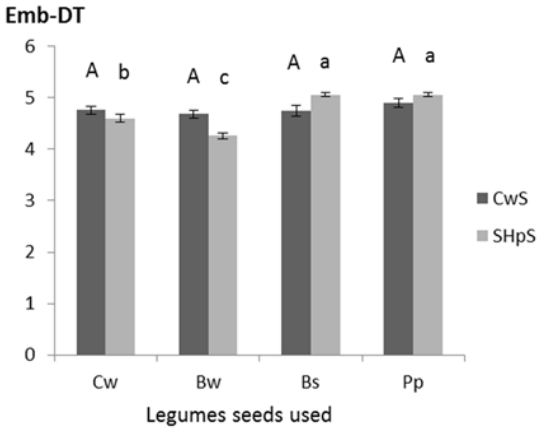

FIGURE 1: VARIATION IN THE EMBRYONIC DEVELOPMENT TIME OF *C. MACULATUS* ON COWPEA SEEDS, WHITE BAMBARA GROUNDNUT, STRIPED BAMBARA GROUNDNUT AND PIGEON PEA (CWS: COWPEA STRAIN; SHPS: SECONDARY HOST PLANT STRAIN). EMB-DT: EMBRYONIC DEVELOPMENT TIME (DAYS); CW: COWPEA SEEDS; BW: WHITE BAMBARA GROUNDNUT; BS: STRIPED BAMBARA GROUNDNUT; PP: PIGEON PEA.
MEANS (± SE) ARE COMPARED AND VALUES WITH DIFFERENT LETTERS DIFFER SIGNIFICANTLY ACCORDING TO FISHER'S LSD TEST AT SIGNIFICANCE LEVEL OF 5%. CAPITAL LETTERS ARE USED FOR THE COWPEA STRAIN AND LOWER FOR STRAINS FROM THE SECONDARY HOST PLANTS.

For *C. maculatus* reared on cowpea, embryonic development time was very similar to those reared on the seeds of the other legumes used. The values obtained from those reared on the cowpea seeds did not differ significantly from those from Bambara groundnut or pigeon peas.

For *C. maculatus* reared on white or striped Bambara groundnut or pigeon pea, embryonic development time varied from one plant to another. The embryonic development time on striped Bambara groundnut and pigeon pea was significantly higher than that of cowpea. However the embryonic development time on cowpea was significantly higher than that of white Bambara groundnut.

3.2 Development time of larval stages

3.2.1 First instar (L1)

For individuals reared on cowpea, development time of first instar varied between the host plant taxa (Fig. 2A). Development time was significantly longer on the striped Bambara groundnut than on white Bambara groundnut and pigeon pea. However, these were significantly longer than development time recorded from cowpea.

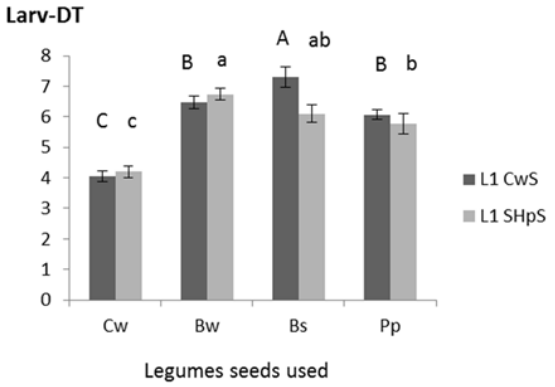

FIGURE 2A: VARIATIONS IN THE DEVELOPMENT TIME OF THE FIRST INSTAR (L1) OF *C. MACULATUS* DEPENDING ON HOST PLANTS (CWS: COWPEA STRAIN; SHPS: SECONDARY HOST PLANT STRAIN). LARV-DT: LARVAL DEVELOPMENT TIME; CW: COWPEA SEEDS; BW: WHITE BAMBARA GROUNDNUT; BS: STRIPED BAMBARA GROUNDNUT; PP: PIGEON PEA. MEANS (± SE) ARE COMPARED AND VALUES WITH DIFFERENT LETTERS DIFFER SIGNIFICANTLY ACCORDING TO FISHER'S LSD TEST AT PROBABILITY LEVEL OF 5%. L1 IS THE FIRST LARVAL STAGE. CAPITAL LETTERS ARE USED FOR THE COWPEA STRAIN AND LOWER FOR STRAINS FROM THE SECONDARY HOST PLANTS.

Regarding *C. maculatus* from the other host plants such as white and striped Bambara groundnut or pigeon pea, the development time of the first instar (L1) was not significantly different. Also, the development time on white Bambara groundnut was significantly different from that obtained on the pigeon pea. Again, these were significantly longer than the development time recorded from cowpea.

3.2.2 Second instar (L2)

For individuals reared on cowpea, the development time of second instar on white Bambara groundnut and pigeon pea was not significantly different (Fig. 2B). However, these times were significantly longer than that of individuals from cowpea and striped Bambara groundnut. The development time on the striped Bambara groundnut was significantly longer than that of cowpea.

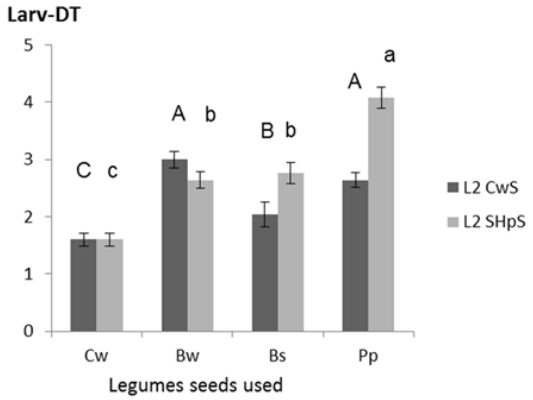

FIGURE 2B: VARIATIONS IN THE DEVELOPMENT TIME OF THE SECOND INSTAR (L2) OF *C. MACULATUS* DEPENDING ON SECONDARY HOST PLANTS (CWS: COWPEA STRAIN; SHPS: SECONDARY HOST PLANT STRAIN). LARV-DT: LARVAL DEVELOPMENT TIME; CW: COWPEA SEEDS; BW: WHITE BAMBARA GROUNDNUT; BS: STRIPED BAMBARA GROUNDNUT; PP: PIGEON PEA.

MEANS (± SE) ARE COMPARED AND VALUES WITH DIFFERENT LETTERS DIFFER SIGNIFICANTLY ACCORDING TO FISHER'S LSD TEST AT PROBABILITY LEVEL OF 5%. L2 CORRESPONDS TO THE SECOND INSTAR. CAPITAL LETTERS ARE USED FOR THE COWPEA STRAIN AND LOWER FOR STRAINS FROM THE SECONDARY HOST PLANTS

For individuals reared on the other host plants, the development times of larval stage L2 were significantly shorter for white and striped Bambara groundnut than that of pigeon pea. The development time of larval stage L2 from cowpea was significantly shorter than that of the two varieties of Bambara groundnut and pigeon pea.

3.2.3 Third instar larvae (L3)

For individuals reared on cowpea, development time on striped Bambara groundnut and pigeon pea were very similar (Fig.2C). However, these development times were significantly longer than on the white Bambara groundnut. Development time on cowpea seeds was significantly longer than on the white and striped Bambara groundnut.

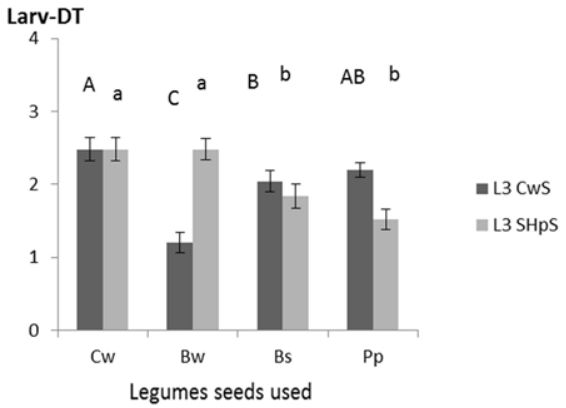

FIGURE 2C: VARIATIONS IN THE DEVELOPMENT TIME OF THE THIRD LARVAL STAGE (L3) OF *C. MACULATUS* DEPENDING ON SECONDARY HOST PLANTS (CWS: COWPEA STRAIN; SHPS: SECONDARY HOST PLANT STRAIN). LARV-DT: LARVAL DEVELOPMENT TIME; CW: COWPEA SEEDS; BW: WHITE BAMBARA GROUNDNUT; BS: STRIPED BAMBARA GROUNDNUT; PP: PIGEON PEA.

MEANS (± SE) ARE COMPARED AND VALUES WITH DIFFERENT LETTERS DIFFER SIGNIFICANTLY ACCORDING TO FISHER'S LSD TEST AT PROBABILITY LEVEL OF 5%. L3 CORRESPONDS TO THE THIRD INSTAR. CAPITAL LETTERS ARE USED FOR THE COWPEA STRAIN AND LOWER FOR STRAINS FROM THE SECONDARY HOST PLANTS.

For individuals reared on other host plants, development time of the third instar (L3) on cowpea and white Bambara groundnut was not significantly different. However, they were significantly longer than development time on the striped Bambara groundnut and pigeon peas.

3.2.4 Fourth instar (L4)

For individuals reared on cowpea, development times of the fourth instar on cowpea, striped Bambara groundnut and pigeon pea were almost identical (Fig. 2D). Development time was significantly shorter than on the white Bambara groundnut.

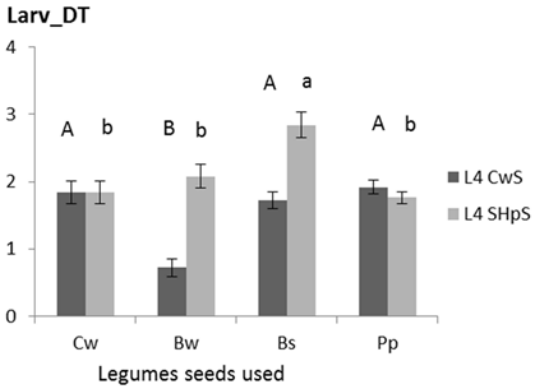

FIGURE 2D: VARIATIONS IN THE DEVELOPMENT TIME OF THE FOURTH LARVAL STAGE (L4) OF C. MACULATUS BASED ON SECONDARY HOST PLANTS (CWS: COWPEA STRAIN; SHPS: SECONDARY HOST PLANT STRAIN). LARV-DT: LARVAL DEVELOPMENT TIME; CW: COWPEA SEEDS; BW: WHITE BAMBARA GROUNDNUT; BS: STRIPED BAMBARA GROUNDNUT; PP: PIGEON PEA.

MEANS (± SE) ARE COMPARED AND VALUES WITH DIFFERENT LETTERS DIFFER SIGNIFICANTLY ACCORDING TO FISHER'S LSD TEST AT PROBABILITY LEVEL OF 5%. L4 CORRESPONDS TO THE FOURTH INSTAR. CAPITAL LETTERS ARE USED FOR THE COWPEA STRAIN AND LOWER FOR STRAINS FROM THE SECONDARY HOST PLANTS.

For individuals reared on other host plants, development times of the fourth instar on cowpea, white Bambara groundnut, and pigeon pea were not significantly different. However, they were significantly longer than on striped Bambara groundnut.

3.2.5 Pupal stage

With individuals reared on cowpea, pupal development times on white Bambara groundnut and pigeon pea were significantly longer than on the striped Bambara groundnut (Fig. 2E). However, the duration of development on cowpea was significantly longer than on the striped Bambara groundnut and pigeon pea.

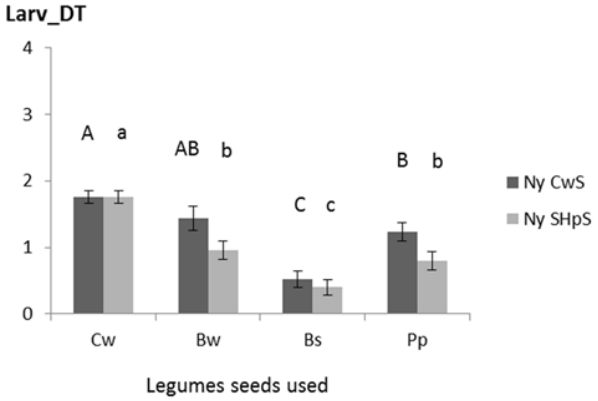

FIGURE 2E: VARIATIONS IN THE DEVELOPMENT TIME OF THE NYMPH (NY) OF C. MACULATUS DEPENDING ON SECONDARY HOST PLANTS (CWS: COWPEA STRAIN; SHPS: SECONDARY HOST PLANT STRAIN). LARV-DT: LARVAL DEVELOPMENT TIME; CW: COWPEA SEEDS; BW: WHITE BAMBARA GROUNDNUT; BS: STRIPED BAMBARA GROUNDNUT; PP: PIGEON PEA.

MEANS (± SE) ARE COMPARED AND VALUES WITH DIFFERENT LETTERS DIFFER SIGNIFICANTLY ACCORDING TO FISHER'S LSD TEST AT PROBABILITY LEVEL OF 5%. CAPITAL LETTERS ARE USED FOR THE COWPEA STRAIN AND LOWER FOR STRAINS FROM THE SECONDARY HOST PLANTS.

For individuals reared on the other host plants, development times of the nymph obtained on white Bambara groundnut, and pigeon pea were not significantly different. However, they were significantly longer than development time on striped Bambara groundnut. Development time on cowpea was significantly longer than on the white and striped Bambara groundnut and pigeon pea. The pupal stage appeared to be the shortest stage in almost all host plants used.

With individuals raised on cowpea, cumulative development times of pupa on white and striped Bambara groundnut were almost identical (Fig. 2F). These durations were significantly shorter than development times on the pigeon pea. However, development time on cowpea was significantly shorter than on the two varieties of Bambara groundnut.

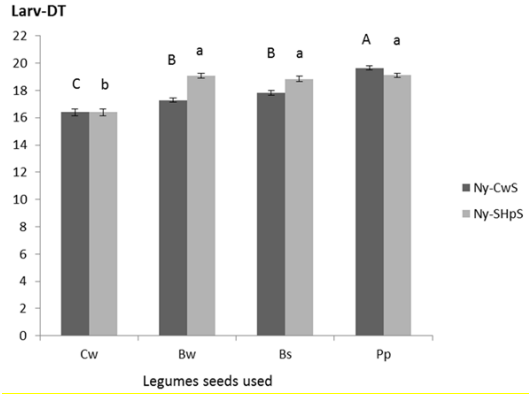

FIGURE 2F: VARIATIONS IN CUMULATIVE DEVELOPMENT TIME OF THE NYMPH (NY) OF *C. MACULATUS* DEPENDING ON SECONDARY HOST PLANTS (CWS: COWPEA STRAIN; SHPS: SECONDARY HOST PLANT STRAIN). LARV-DT: LARVAL DEVELOPMENT TIME; CW: COWPEA SEEDS; BW: WHITE BAMBARA GROUNDNUT; BS: STRIPED BAMBARA GROUNDNUT; PP: PIGEON PEA.

MEANS (± SE) ARE COMPARED AND VALUES WITH DIFFERENT LETTERS DIFFER SIGNIFICANTLY ACCORDING TO FISHER'S LSD TEST AT PROBABILITY LEVEL OF 5%. NY CORRESPONDS TO THE PUPAL STAGE. CAPITAL LETTERS ARE USED FOR THE COWPEA STRAIN AND LOWER FOR STRAINS FROM THE SECONDARY HOST PLANTS.

For individuals reared on the other host plants, cumulative development times of pupa on white Bambara groundnut, striped Bambara groundnut and pigeon pea were not significantly different. However, these durations were significantly longer than development time on cowpea.

IV. DISCUSSION

The embryonic and larval development of *C. maculatus* may occur at different speeds indifferent secondary host plants. Indeed, there was no difference between the embryonic development times on seeds of secondary hosts for individuals raised on cowpea. However, those that were raised for two years on secondary host plants had a significantly slower embryonic development time on white Bambara groundnut seeds in comparison with cowpea seeds. Embryonic development in *C. maculatus* occurs inside the egg which is attached to the seed. The embryo has no contact with either the outside environment or with the interior of the seed, therefore only the seed coat influences embryonic development. From then on, the physical (structure) and chemical (substances emitted) properties of the seed coat therefore would influence the embryonic development time.

Larval development time varies from one instar to another, independent of the type of seeds of the plants used in the study. For the first (L1) and second (L2) instars, the larval development time of *C. maculatus* was significantly longer on pigeon pea and Bambara groundnut than on cowpea regardless of the strain of *C. maculatus* used. The long development time observed in those individuals reared on the secondary host plants in general and pigeon peas in particular could be explained by an abundance of noxious substances and/or antifeedants contained in the seeds. These substances would negatively impact the immature stages of *C. maculatus,* causing significant larval mortality or prolonging their development, which was observed in our case. These results agree with those obtained by several authors. [5]suggest that the development time of weevils could be influenced by chemical substances in the seeds of host plants. According to[7], *C. maculatus* would have difficulties growing within *Calopogonium mucunoides* Desv., *Desmodium intortium* Urb., and *Centro pubesens* Benth. because of the presence of toxic substances in the seeds. However, within the seeds of some less common host plants such as *Flemingo congesta* Vest., it would be possible to complete its development cycle, but with a longer post-embryonic development time.

Another factor that could influence the larval development may be intraspecific competition among larvae inside the seeds. Females have the ability to lay several eggs in each seed [23]. The larvae which develop inside the seeds cannot move between seeds. The seed is a limited food resource and ensuing competition between intraspecific larvae inside the seeds could negatively affect larval development [24]. Our results also show that individuals raised on pigeon pea have a slower development time than those raised on cowpea. This may be linked to the process of adaptation. Adaptation to specific habitats affects the evolution of life history traits of organisms by optimizing the efficiency of these organisms to exploit the resources of their environment. Although several associations between phytophagous insects and their host plants are highly conserved [25], there are several examples of insect populations, which adapt quickly to new host plants [26]. According to [27], if a population has a particular way of detoxifying a new secondary host compound, it may be able to exploit closely related hosts of the same family that contain similar compounds. According to [28], when a population of an insect herbivore encounters a plant species with evolutionarily unfamiliar secondary metabolites, there are many possible outcomes, including:

- No development is possible as the insect's biochemical mechanisms are unable to process the unfamiliar secondary metabolites (i.e. no host shift).
- Most, if not all, individuals in the population are able to develop on the new host species either as well as, or slower than it would on the primary host species. This is because the insect's generalized biochemical mechanisms are robust enough to be able to process the unfamiliar secondary metabolites. This seems to be the case regarding the response of *C. maculatus* to pigeon pea in this study.

[29]have also shown that *C. maculatus* can adapt to new marginal hosts such as *Lens culinaris* Medikus, which is a plant whose seeds are as hard as those of pigeon pea.

Lastly, the pupal stage seems to be the shortest stage of all the immature stages observed, and this is more pronounced for nymphs from secondary host plants. At this stage, the maximum size is reached. The pupae do not eat from the seed but uses its nutritious reserves stored during larval development [22;21]. This would explain the very limited influence of the host seed on this stage.

V. CONCLUSION

This study allowed us to demonstrate that embryonic and larval development time of *C. maculatus* can be prolonged by the use of secondary host plants. Indeed, the results showed that development time of the two early instars for all strains was significantly longer on the three secondary host plant seeds than on cowpea seeds.

Furthermore, for *C. maculatus* reared on white Bambara groundnut, embryonic development time on white Bambara groundnut was significantly lower than that on cowpea and pigeon pea. Thereby, it appears that the prolonged development within the seeds of a plant host, influence the embryonic of *C. maculatus* on this plant. So, *C. maculatus* has a potential to become a serious pest for Bambara groundnut, especially when it is exposed to the seeds of this plant for a relatively long time. However, even though cowpea and Bambara groundnut belong to the same genus, the cumulative duration of development of the nymph of *C. maculatus* on cowpea is short compared to that obtained on the two varieties of Bambara groundnut even after two years of development or adaptation of these plants. Pigeon pea provided the worst conditions for larval and pupal instar development. These results lead to better understanding of post-embryonic development of *C. maculatus* occurring within the seeds of secondary host plants. They could be used in the context of the development of adequate methods of pest control; especially for the development of an integrated pest management program against the Bruchidae in West Africa.

ACKNOWLEDGEMENTS

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