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Identification of Two Aphids' (*Aphis craccivora* Koch) Biotypes Prevailing in Three Cowpea (*Vigna unguiculata* (L). Walp.) Growing Areas in Burkina Faso

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ABSTRACT

Cowpea (*Vigna unguiculata* (L). Walp.) is a plant-based protein crop with high nutritional value but highly susceptible to insect pests, particularly aphids (*Aphis craccivora* Koch). Aphids can destroy 80 to 100% of cowpea farms and also transmit viral diseases like cowpea mosaic virus, significantly reducing yields. This study aimed to investigate the evolution of aphid infestations from three cowpea-growing locations of Burkina Faso (*Bobo*, *Kamboinsé*, and *Pobé*) and identify the prevailing aphid biotypes. Therefore, ten varieties were screened with three aphids' strains from these locations. A randomized complete block design

with three (3) blocks and five (5) repetitions each was used. The number of leaves per plant, the survival of individual plant and the degree of infestation were evaluated. Results revealed that the degree of infestation used as a criterion for evaluating cowpea resistance to aphids is just as reliable during the early stages of plants development as during the following stages. Two biotypes of aphids have been identified. Indeed, the *Kamboinse* strain is similar to that of *Pobé* and these two strains are significantly different from the strain of *Bobo*. The close relationship between the 2 biotypes suggests the possibility to develop varieties strong enough to resist to both of them. A molecular characterization of the three aphids' strains would provide more precision on the genetics of the two aphides' biotypes.

Keywords: *Vigna unguiculata*, *Aphis craccivora*, biotypes.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L). Walp.) is a protein-producing plant with high nutritional value but very prone to insect attacks. With a protein content of over 25% (Boukar *et al.*, 2011), the crop is an alternative to animal-based proteins (Gil *et al.*, 2024) and supplements the nutritional intake of low-protein dishes such as those based on cereals and tubers. Cowpea adapts well to different types of soils, tolerates drought and can be used to improve soil fertility.

Although Burkina ranks third in the world in terms of production (FAOSTAT, 2020), yields remain low. The major biotic stress affecting cowpea yields concerns harmful insects, including aphids(*Aphis craccivora* Koch), which can destroy 80 to 100% of cowpea farms (Singh and Jackai 1985). Aphids can also transmit viral diseases, such as cowpea mosaic virus (CBMV), which significantly reduces cowpea yield in farmers' fields (Aliyu *et al.*, 2023). Additionally, aphids honeydew induce sooty molds grow on leaves and hinder the photosynthetic activities of plants (Guo *et al.*, 2022). With their high reproductive potential and their phenotypic plasticity, aphids are among the most destructive legume insect pests in the world (Wongsa et al. 2017; Guo et al. 2022). Therefore, controlling these phytophagous insects is a factor in increasing yields. And, to achieve this, the use of resistant cultivars is the cheapest and most effective way (Nualsri et al. 2012). However, although the development and deployment of aphid-resistant cowpea cultivars is an environmentally friendly way to combat this pest, its effectiveness depends on the stability of the resistance. Hence the need to know the biotypes that evolve in the growing areas. The general objective of this study is to contribute to improving cowpea yields through a better knowledge of the aphid biotypes prevailing in cowpea growing areas in Burkina Faso. In order to achieve this, two specific objectives have been identified: (i) investigate the evolution of the infestation of 3 strains of aphids from 3 cowpea growing areas, (ii) identify aphids' biotypes prevailing in these three growing areas.

MATERIALS AND METHODS

The activity was carried out at the Institute of Environment and Agricultural Research (*INERA*), which is one of the four institutes of the National Centre for Scientific and Technological Research (*CNRST*). The different strains of aphids harvested were reared at the Laboratory of Entomology and the screening were carried out at the Genetics and

Biotechnology laboratory based at the Environmental, Agricultural Research and Training Centre (*CREAF*) of *Kamboinsé.* The geographical coordinates of the research station are 12°28 North latitude, 1°32 West longitude and 296 meters above sea level.

Three strains of aphids harvested on cowpea plants in the West (*Bobo*), the Centre (*Kamboinsé*), and the North (*Pobé*) of Burkina Faso located in the three different agroecological zones were used for screening. The aphids were harvested in August 2013 and kept on the variety KVx396-4-5-2D for maintaining and rearing.

A no-choice settling test had been performed. Thusly, the plants remained under infestation for the duration of the test as described by Ouédraogo et al. (2018). A randomized complete block design with three (3) blocks and five (5) repetitions each was used. Seven-days-old individual plants were screened. In each block cowpea plants were infested with only one of the three (3) strains of aphids.

Observations were made every three days and consisted of measuring: • The number of leaves; • The survival of individual plants; • The degree of infestation by counting aphid individuals using six scores ranging from 0 to 5: (0 = no aphids, 1 = 1 to 5 aphids, 2 = 5 to 20 aphids, 3 = 20 to 100 aphids, 4 = 100 to 500 aphids, 5 = + 500 aphids).

Raw data of one month after infestation, 20 DAS (Days After Sowing), 35 DAS, 50 DAS and 65 DAS were used for analyses. XLSTAT 7.1 and R version 4.2.3 (R Core Team, 2023) were used to compute the data.

RESULTS AND DISCUSSION

Trend of Aphids' Strains After Infestation

The evolution of aphids' infestation from the day of infestation to 65 days after sowing is displayed in Figure 1. For all the three aphids' strains an exponential increase of the number of aphids is observed from the day of infestation up to 20 days (DAS) after sowing. From 20 DAS to 65 DAS a slight and constant increase in the number of aphids is observed. This result suggests that cowpea, throughout its life cycle, can be a host plant for aphids if they have no alternative. It also shows that the degree of infestation used as a criterion for evaluating resistance is just as reliable during the early stages of the plant development as during the following stages. such method for evaluating cowpea resistance to aphids at the early stage has already been used by Ombakho et al. (1987) and Kim et al. (2008). The method consisted of infesting three-day-old plants and evaluating the resistance of each variety ten days after infestation. The advantage of evaluating resistance at the early stage is that it is faster and less expensive. Aphids can colonize cowpea crops at early stage. In fact, aphids (*alatae*) disperse soon after the rains start, and has been observed to have already colonized the cowpea crops as early as 3 weeks after germination (Ofuya, 1991). It is this ability to rapidly exploit the ephemeral habitats that make aphids serious pests. This ability results from their high reproductive potential, their dispersal abilities and their adaptability to local survival (Dedryver et al. 2010). Results also show that the multiplication of the *Bobo* aphids' strain is faster than those of *Kamboinse* and *Pobe*. This suggests the presence of at least two biotypes. Niassy et al. (1987) reported variable responses in the fecundity and honeydew production of two distinct biotypes of *Schizaphis graminum* (the wheat aphid) on wheat genotypes. Aphid biotypes often interact differently with host plant varieties. Such interactions can lead to a reduction or even a loss of resistance in resistant lines, or an alteration of the insect's bionomics (Annan et al. 1997).

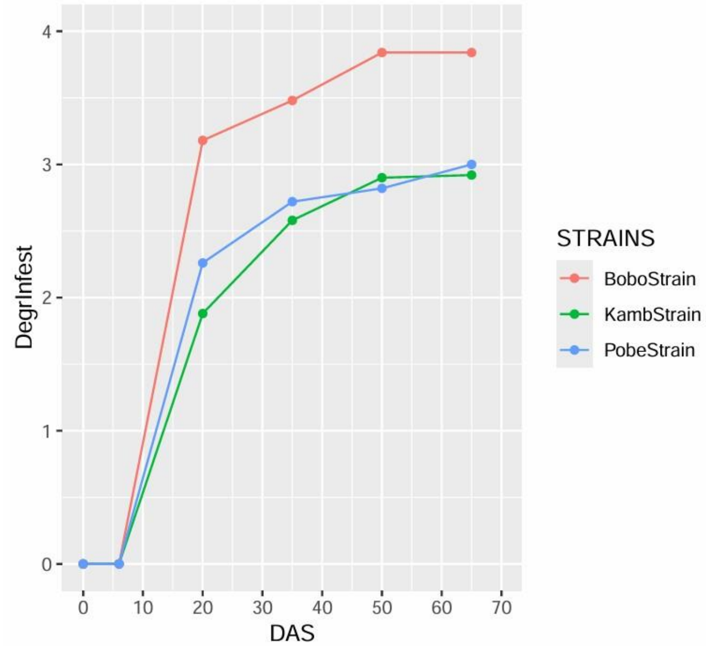

Figure1: Evolution of the average degree of infestation
DegrInf=Degree of infestation; DAS=Number of days after sowing; BoboStrain=Aphids' strain of Bobo; KambStrain=Aphids' strain of Kamboinse; PobeStrain=Aphis strain of Pobe

Characterization of Aphids' Strains

The Discriminant Analysis of the 3 strains of aphids thirty (30) days after infestation gave the results in Table 1, Table 2, Table 3 and Figure 2. The F value associated with the squared Mahalanobis distances between groups (Table 1) shows that the aphids' strain of *Bobo* is significantly different from those of *Kamboinsé* (distance = 3.46) and *Pobé* (distance = 4.20). the P-values (table 2) are respectively 0.019 and 0.008. This result shows the existence of two biotypes among the three strains of aphids. The aphids' strain of *Bobo* constitutes a distinct biotype from that of *Kamboinse* and *Pobé*. In fact, it has been reported a lot of variability within cowpea aphid strains, resulting in existence of several ecotypes or biotypes (Loxdale et al, 2020). The development of aphids' biotypes capable of overcoming resistant varieties complicates the deployment of varietal resistance as an effective management tool (Bhusal et al. 2021). Indeed, many sources of aphid resistance have been identified, several have been deployed. However, some resistances breaking down have been observed (Boissot *et al.*, 2016). Management strategies such as the development of insect-resistant varieties and the application of various insecticides and pesticides in agroecosystems are thought to be at the origin of the intensity of selection pressure (Taggar et al. 2017). These selection pressures and divergent selection in insects, lead to ecological adaptations (Ferrari et al. 2008; Carletto et al. 2009), leading to phenotypic and genotypic differences between insects populations. Thus, for some pests, decisions regarding the deployment of resistance must take into account the genetic structure of the pest population at the regional scale(Thomas *et al.*, 2016). The result also shows that the aphids' strain of *Kamboinse* and the one of *Pobe* are not significatively different (distance=0.24 for a P-value=0.859). Similar results have been found on the geographical distribution of soybean aphid biotypes. Indeed, a large-scale study of soybean aphid biotypes revealed that specific biotypes are not always limited to specific geographical locations, but can be widely distributed (Cooper et al. 2015;Crossley, 2015; Alt and Ryan 2019). On the other hand, it is also possible to find up to three different aphids' biotypes in the same field (Bhusal, Koch and Lorenz, 2021). It is therefore important not only to have a large study area but also to increase the number of samples per collection area.

Figure 2 representing the 3 aphids' strains shows an overlapping distribution, indicating that even though the aphids' strain of *Bobo* is significatively different from those of *Kamboinse* and *Pobe*, there is a close relationship between them, suggesting the possibility to developpe resistant varieties strong enough to resist to both biotypes. In fact, varieties that are resistant to more than one biotype have already been developed (Omoigui et al. 2017; Mofokeng and Gerrano 2021). A genotypic characterisation of the three aphids' strains will help to understand how far the two biotypes differ from one to another. And in the perspective of integrated pest management, aphids' populations with dominant avirulent genes can be released into populations with virulent-recessive genes, which could lead to the insect control by producing biotypes with dominant avirulence genes after a few generations (Foster, and Gallun 1973; Foster and Lafayette 1976).

Table 1: F associated with the squared Mahalanobis distances between groups

	BoboStrain	*KambStrain*	*PobeStrain*
BoboStrain	0.00		
KambStrain	**3.46**	0.00	
PobeStrain	**4.20**	0.25	0.00

BoboStrain=Aphids' strain of Bobo; KambStrain=Aphids' strain of Kamboinse; PobeStrain=Aphis strain of Pobe

Table 2: p-values of F

	BoboStrain	*KambStrain*	*PobeStrain*
BoboStrain	1		
KambStrain	**0.019**	1	
PobeStrain	**0.008**	0.859	1

BoboStrain=Aphids' strain of Bobo; KambStrain=Aphids' strain of Kamboinse; PobeStrain=Aphis strain of Pobe

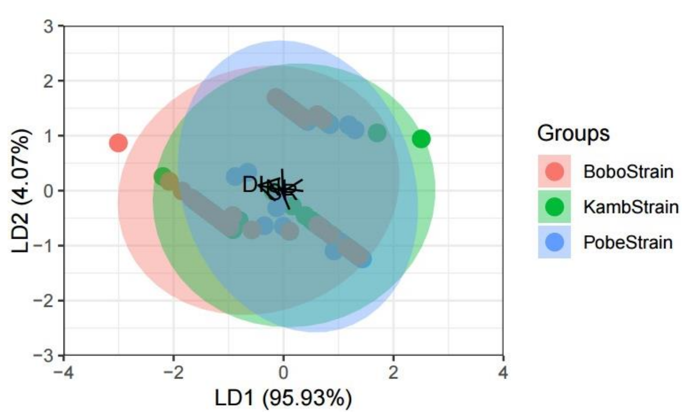

Figure2: Distribution of the 3 aphids' strains

BoboStrain=Aphids' strain of Bobo; KambStrain=Aphids' strain of Kamboinse; PobeStrain=Aphis strain of Pobe

The characteristics of each aphids' strain are displayed in table 3. This result shows that the aphids' strain of *Pobe* has been the most severe in reducing plants growth and survival (NL=8.14; SR=62%). In addition to that, despite the highest degree of infestation (DI=3.84) recorded by the aphids' strain of *Bobo*, its damage on plants growth and survival (NL=10.2; SR=74%) is lower than those of *Kamboinse* (NL=8.58; SR=70%) and *Pobe* (NL=8.14; SR=62%). These results raised questions about how far the number in aphids' population affects plant survival and growth. In fact it is known that the DI is negatively correlated to plant growth and survival (Ouédraogo *et al.*, 2018). Aphids' damage to plants is caused by both nymphs and adults (Ofuya, 1997) and infested plants become yellowish, stunted, and may become unproductive due to direct feeding of sap and toxic salivary secretions of the insect (Whitworth, R. J. and Aqeel, 2009). The present result in accordance with Boissot et al. (2016), shows that depending on the aphids' biotype and the type of varieties, the deleterious effects of aphids' infestation on the plant can be drastically reduced, because the response of aphid populations to the selection pressure exerted by a resistant variety depends on their genetic composition, under the influence of the agrosystem.

Table 3: Description of aphids' strains

strain	PlFr	NL	DI	SR
BoboStrain	50	**10.20**	**3.84**	**74**
KambStrain	50	8.58	2.74	70
PobeStrain	50	8.14	2.84	62

PlFr: Infested plants frequency; NL=Number of leaves; DI=Degree of infestation; SR=Plants survival rate
BoboStrain=Aphids' strain of Bobo; KambStrain=Aphids' strain of Kamboinse; PobeStrain=Aphis strain of Pobe

CONCLUSION

Aphis craccivora Koch is one of the insects responsible for yield reductions in cowpea. The study of the evolution of the infestation showed that the degree of infestation is a reliable criterion for evaluating the resistance of cowpea to aphids during the first phases of plant development and during the following phases as well. The characterization of the 3 aphids' strains established that the aphid strain of *Pobe* is similar to the one of *Kamboinse* and that these two strains are different from that of *Bobo*, what shows the presence of two distinct biotypes among the three aphids' strains. The close relationship between the 2 biotypes suggests the possibility to develop varieties strong enough to resist to both of them. We recommend a molecular characterization of the three aphids' strains in order to provide more precision on the genetics of the two biotypes.

Then, to have an overall view of the biotypes of aphids evolving in the cowpea growing areas, it would be necessary to increase not only the number of study areas but also the number of aphid samples collected per study area for much greater precision. Knowledge of the two biotypes of aphids thusly characterized will allow a better deployment of resistant cowpea varieties once developed.

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