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Impact of predation by coccinellid larvae on colonies of the mealybug *Phenacoccus manihoti* in crop lands

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Abstract

A field study of predation by coccinellid larvae (Col. Coccinellidae) on populations of cassava mealybugs *Phenacoccus manihoti* Matile-Ferrero (Hom. Pseudococcidae) was made at two localities in the Congo, with an indigenous species *Exochomus flaviventris* Mader and an exotic one, *Hyperaspis raynevali* Mulsant.

H. raynevali larvae were released on cassava plants on 7 August on 17±4 mealybugs/plant and on the 5 September on 89±25 mealybugs/plant. The releases of *E. flaviventris* larvae occurred on 17 August with 19±7 mealybugs/plant and on 6 October with 72±33 mealybugs/plant. 4 weeks after the first introducton of *H. raynevali*, the numbers of the pest multiplied by 5 on the treated plants and by 12 on the controls. In parallel, *E. flaviventris* larvae maintained the *P. manihoti* populations below the level of 20 mealybugs/plant for 3 weeks, whereas on control plants their number increased by 3.3. It was not possible, however, to obtain reliable data for the second release, because it coincided with a decline in mealybug populations attributable to natural regulatory phenomena (local *E. flaviventris*, rain, physiological state of the host-plant). The impact of *E. flaviventris* and *H. raynevali* was influenced by the density and age structure of the prey, but the *predatory* potential of the two coccinellids could not be compared.

Keywords: *Hyperaspis raynevali*, *Exochomus flaviventris*, Coccinellidae, *Phenacoccus manihoti*, cassava, predatory impact, Congo.

Résumé

Une étude de l'impact prédateur des larves d'*Exochomus flaviventris* Mader (*Col. Coccinellidae*) espèce indigène et d'*Hyperaspis raynevali* Mulsant (*Col. Coccinellidae*) espèce exotique sur les colonies de la cochenille du manioc *Phenacoccus manihoti* Matile-Ferrero (*Hom. Pseudococcidae*) a été entreprise au Congo dans deux parcelles paysannes.

Les larves d'*H. raynevali* sont lâchées, le 7 août, sur des effectifs de 17±4 cochenilles/plante et le 5 septembre sur des effectifs de 89±25 cochenilles/plante. Les lâchers de larves d'*E. flaviventris* interviennent le 17 août sur des effectifs de 19±7 cochenilles/plante et le 6 octobre sur des effectifs de 72±33 cochenilles/plante. Quatre semaines après le premier lâcher d'*H. raynevali*, les effectifs du ravageur sont multipliés par 5 sur les plants traités et par 12 sur les plants témoins. Parallèlement, les larves d'*E. flaviventris* maintiennent les effectifs de *P. manihoti* en dessous de 20 cochenilles/plante pendant 3 semaines, alors que sur les plants témoins ils sont multipliés par un facteur 3,3. Le 2e lâcher n'a pas permis d'obtenir des résultats fiables car il est intervenu simultanément avec les facteurs de régulation naturels responsables de la chute de populations de cochenilles. Nos résultats de terrain montrent que l'impact prédateur des larves d'*H. raynevali* et d'*E. flaviventris* est influencé par la densité et la structure d'âge des populations de cochenilles. Ils ne permettent pas d'établir une comparaison stricte entre les potentialités prédatrices des deux espèces de coccinelles.

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INTRODUCTION

Originating in the tropical regions of South America, the mealybug *Phenacoccus manihoti* Matile-Ferrero (Hom. Pseudococcidae) was reported for the first time on the African continent in 1973, in the Congo and in Zaire (SILVESTRE, 1973; HAHN & WILLIAMS, 1973). By 1986, this pest had spread across 25 countries situated in the cassava growing areas of Africa (NEUENSCHWANDER & HERREN, 1988) causing losses evaluated at between 30 and 84% (NWANZE, 1982).

From 1977 onwards, several species of entomophagous insects have been collected in South America, for the purpose of biological control of the mealybug in Africa (YASEEN & BENNETT, 1979). In the Congo, *Epidinocarsis lopezi* De Santis (Hymenoptera, Encyrtidae), has become acclimatised since 1982, but has not significantly affected cassava mealybug populations (IZIQUEL & LE RÜ, 1989; LE RÜ *et al.*, 1991). Although exotic or indigenous Coccinellidae predators (*Diomus sp.*, *Hyperaspis spp.* and *Exochomus spp.*) have been recognised as agents capable of reducing the populations of *P. manihoti* in Nigeria (LEMA & HERREN, 1983), in Zaire (HENNESSEY & MUAKA, 1987) and in the Congo (IZIQUEL & LE RÜ, 1989), no reports of experimentation quantifying their effects have been published.

The purpose of our study was to evaluate the impact of experimentally introduced coccinellid larvae on the population dynamics of *P. manihoti*. At the same time, a parallel study was carried out on the exotic coccinellid *Hyperaspis raynevali* Mulsant (Coleoptera, Coccinellidae, syn. *jucunda*) (NSIAMA SHE, pers. comm.) and on a local ladybird beetle, *Exochomus flaviventris* Mader (Coleoptera, Coccinellidae) considered to be the most active predator of the cassava mealybug in the Congo (FABRES & KIYINDOU, 1985; IZIQUEL & LE RÜ, 1989).

MATERIAL AND METHODS

Experimentation lasted from July to November 1989, during the 5 months generally corresponding to the multiplication period of *P. manihoti*, in the region of Brazzaville (Pool). It was conducted on two crop lands, each covering 1,000 m^2, one situated at Brazzaville (Centre ORSTOM), to study the indigenous ladybird *E. flaviventris*, and the other at Kombé (17 km south-west of Brazzaville) to observe the exotic predator *H. raynevali*. These plots were planted with Cassava *Manihot esculenta* Crantz (Euphorbiaceae), crops of the M'Pembé variety, at a density of one cutting per square meter. The plants were 10 to 12 months old at the time of the investigation and measured 1 to 2 m high. The test was carried out on cassava stems having no point of contact with other shoots. The crops were left untended, apart from weeding.

At the end of the rainy season (late May), two groups of 30 cassava plants, devoid of mealybugs, were prepared on each plot: one being the control group (C), on which the mealybug populations evolved in the presence of natural regulation factors only, and the other, group (T), "treated" with *H. raynevali* or *E. flaviventris* larvae according to the locality, where the evolution of mealybug colonies, into which predators have been introduced, could be followed. The plants were evenly distributed on each plot of land.

In the Congo, the population dynamics of *P. manihoti* varies considerably from one field to another (LE RÜ *et al.*, 1991). For a comparative study between two localities, conditions must be as much alike as possible. The two cassava crops were therefore artificially infested at the start of the dry season (6 and 7 June 1989) by attaching fragments of the cassava stem colonised by *P. manihoti* to about ten cassava plants, distributed at regular intervals in each plot of land. Those cassava plants were not followed during the experimentation.

Coccinellids at the 2nd instar were chosen for release, as they then have the dual advantage of being easy to handle in the field, while possessing, prior to nymphosis, a high trophic potential (REYD

et al., 1991). Thus the cumulative amount of food consumed by *H. raynevali* in the course of the 2nd, 3rd and 4th larval instars is 17 mg of cassava mealybug (equivalent in weight to 11 mealybugs at the 4th instar), while that of *E. flaviventris* is 71 mg (43 mealybugs at the 4th instar, KANIFA-KIAMFU, unpubl.). These values correspond to 97% of the total amount of food consumed during the whole pre-imaginal development period for both ladybird species.

A large number of 2nd instar predator larvae was needed to carry out the biological treatments. To procure them, we used a method based on the fact that the coccinellids' favourite site for oviposition is the mealybug ovisac. About ten ovisacs were removed from female *P. manihoti* and then fixed individually on strips of card (2.5 × 70 mm). These supports with deposited eggs were placed in transparent plastic tubes covered with wire mesh at the opening (L = 97 mm, ∅ = 28 mm), in the presence of adult coccinellids, in the following conditions: T = 25 ± 2°C, H.R. = 60-80%, Photophase = 12 hours. In each tube 10 females and 5 males of *E. flaviventris* or *H. raynevali* were placed for 36 hours, then removed to prevent them from eating their own eggs. After incubation, the larvae cohort thus obtained could be used directly in the field, four or five days later.

Releases of coccinellid larvae were carried out in each locality, at two densities of the prey. Dates of treatments were determined by the mean density of mealybugs/stem, regardless of the age structure of the pest populations. Indeed it is impossible in open-air conditions to control simultaneously the density and the age-structure of the mealybug populations. *H. raynevali* larvae were released on 7 August on 17 ± 4 mealybugs/plant and on 5 September on 89 ± 25 mealybugs/plant. The releases of *E. flaviventris* larvae occurred on 17 August with 19 ± 7 mealybugs/plant and on 6 October with 72 ± 33 mealybugs/plant.

During the biological treatments, the number of coccinellid larvae attached to each stem was proportional to the total number of mealybugs counted on a given day (all instars together). The larvae were placed with a fine brush close to the mealybug colonies. During the first release, the prey/predator ratio was 3/1 for densities of around 20-25 mealybugs/plant (start of infestation). This ratio was 7/1 in the course of the second release for initial densities of about 70-80 mealybugs per plant (colonies in rapid growth phase). In the second case, the prey/predator ratio was much higher, as the coccinellid larvae production was then lower. These proportions are very similar to those used with aphidophagous coccinellid species in greenhouses, to combat *Mysus persicae* Sulz (Hom. Aphididae) (IPERTI & QUILICI, 1984).

The coccinellid larvae deposited on the cassava plants exercised predation from the 2nd larval instar until nymphosis. As soon as they appear, the pre-pupae or pupae were all collected, to prevent the adult ladybirds from the "treated" plants from colonising the "control" plants.

Weekly visual counting was made on these plants between 8 and 11 a.m. The mealybugs were counted, their larval instar (L1 + L2 + L3) or adult development stage (immature females or with ovisac) recorded, as well as their state, alive or dead (killed by the entomophagous fungus *Neozygites fumosa* (Speare) Remaudière & Keller, or parasitised by *E. lopezi* De Santis). The percentages of mealybugs, parasitised or having died from mycosis, were calculated with regard to the total number of mealybugs, whatever their instar. The presence of other entomophagous species associated with *P. manihoti* are also noted.

In the text the means of the numbers of mealybugs per stem are given with a confidence margin $t.s/\sqrt{n}$, (s) being the standard deviation, (n) the number of plants per batch. The general evolution of the numbers of mealybugs was statistically compared with a t-test after a logarithmic transformation of the data. We test the hypothesis that the slope of the straight regression line calculated between the transformed data of the two groups of plants at each moment, shows a significant difference of 1, with t the value read in the table for d.d.l. $= n - 1$ for 5% level of significance.

Climatic data were provided by the ORSTOM meteorological stations at Brazzaville and the Centre National des Semences Améliorées (National Centre for Seed Improvement) at Kombé. The mean rainfall and temperature means for 2 weeks for both localities are given in table I.

TABLE I. – *Meteorological conditions in the experimental areas. Mean temperature and rainfall every 2 weeks.*

	July		August		September		October	
Kombé								
Temperature (°C)	21.8	21.4	24.4	24.5	25.8	26.3	25.1	27.9
Rainfall (mm)	0	0	0	0	0	71	60	133
Brazzaville								
Temperature (°C)	22.5	22.3	23.7	25.4	26.0	26.0	25.5	26.1
Rainfall (mm)	0	0	3	1	2	36	44	172

RESULTS

Influence of local natural enemies

Local species of natural enemies have been observed on the experimental plots in the course of the study: predators are *E. flaviventris* and *Hyperaspis senegalensis* Mulsant (Col. Coccinellidae), *Spalgis lemolea* Druce (Lep. Lycaenidae), the parasitoid *E. lopezi* and the entomophthorale *N. fumosa*.

At Kombé and Brazzaville respectively, the ladybird *E. flaviventris* accounts for 90 and 98% of the total number of predators encountered in the course of the dry season on the control plants. Abundance of local natural enemies on the day

TABLE II. – *Abundance of local natural enemies of* P. manihoti *on* 30 *test plants at the date of release* (*L: larvae, P: pupae, A: adults of* E. flaviventris).

	Number of *E. flaviventris*		Mummies %		*P. manihoti* killed by *N. fumosa*	
	First release	Second release	First release	Second release	First release	Second release
Kombé						
Control plants	0	5 L+2 P+2 A	0.2	0.08	0	0
Test plants	0	5 L+3 A	0.01	0.02	1.6	0
Brazzaville						
Control plants	2 L+1 P+11 A	58 L+6 P+20 A	1.6	3.7	1.5	0.1
Test plants	2 L+6 A	28 L+2 P+39 A	2.7	1.2	1.1	0

of each release is given in table II. The presence of other predators is limited to a few individuals, observed above all when infestation is ending. The percentages of mummies recorded during the two releases, on test plants, did not exceed 2.7 and 1.2% at Brazzaville. Only a few mummies were recorded on test plants, during the two releases, at Kombé. The percentage of mealybugs killed by the entomopathogenic *N. fumosa* during the first release is respectively 1.6% at Kombé and 1.1% at Brazzaville. No individuals which had died from mycosis were observed during the second release in either locality.

Impact of the releases of *H. raynevali* and *E. flaviventris*

Data on the density of released and local predators, on test and control plants, are given on table III. At Kombé, no *E. flaviventris* was observed during the first

TABLE III. – *Evolution of the numbers (on 30 test plants) of* H. raynevali *(Kombé) and* E. flaviventris *(Brazzaville) after the two releases (L: larvae, P: pupae, A: adult).*

	First release			Second release		
	J	J+7	J+14	J	J+7	J+14
Kombé						
Test plants						
H. raynevali released	186 L2	92 L4	48 P	355 L2	181 L4	34 P
Local *E. flaviventris*	0	0	0	5 L+3 A	7 L+2 P+2 A	2 L+4 P+5 A
Control plants:						
Local *E. flaviventris*	0	0	0	5 L+2 P+2 A	6 L+4 P+1 A	3 L+4 P+4 A
Brazzaville						
Test plants						
E. flaviventris released	192 L2	69 L4	29 P	338 L2	154 L4	34 P
Local *E. flaviventris*	2 L+6 A	7 L+2 P+7 A	8 L+2 P+6 A	28 L+2 P+39 A	34 L+7 P+27 A	26 L+13 P+35 A
Control plants						
Local *E. flaviventris*	2 L+1 P+11 A	9 L+1 P+10 A	10 L+2 P+7 A	58 L+6 P+20 A	65 L+18 P+23 A	34 L+15 P+37 A

release, and less than 10 (larvae + adult) during the second release. At Brazzaville, on the first day of the releases, local *E. flaviventris* were 24 times (first release) and 5 times (second release) less numerous than the released ones.

After the release of *H. raynevali* larvae, the general evolution of the numbers of mealybugs during the observation period differs significantly between the treated and the control plants (d.d.l. = 11, $_{\text{calculated}}t = 5.029 > \text{to}\ _{\text{theoretical}}t = 2.201$) (fig. 1 A).

4 weeks after the first introduction of *H. raynevali*, the numbers of the pest had multiplied by 5 on the treated plants and by 12 on the control plants. Following the second release, the numbers of mealybugs passed within a week from 89 ± 25 to 82 ± 21 mealybugs/plant on the treated plants and from 227 ± 58 to 259 ± 77 mealybugs/plant on the control plants. After that, multiplication of the mealybugs is comparable on both groups of plants.

After the release of *E. flaviventris* larvae the general change in the numbers of mealybugs during the observation period differs significantly between the treated and the control plants (d.d.l. = 14, $_{\text{calculated}}t = 8.804 > \text{to}\ _{\text{theoretical}}t = 2.145$) (fig. 1 B). Following the first release, the *E. flaviventris* larvae maintain the *P. manihoti* populations below the level of 20 mealybugs/plant for 3 weeks, whereas on the control plants, during the same period, the number of pests multiplies by 3.3. The second release did not modify the evolution of the numbers of *P. manihoti* in the treated plants, in comparison with that of the control plants.

Figure 2 presents variations in the numbers of *P. manihoti* in the larval or adult stages, in the presence and in the absence of *H. raynevali* larvae (fig. 2, A and B) and of *E. flaviventris* (figs. 2 C and D). On both plots of land, the general evolution of the numbers of mealybug larvae during the observation period differs significantly between the treated and the control plants ($_{\text{calculated}}t = 4.724$ with d.d.l. = 11 and $_{\text{calculated}}t = 7.360$ with d.d.l. = 14 at Kombé and at Brazzaville respectively). Regarding the *P. manihoti* adults, a similar statistical test shows that the evolution of their numbers differs significantly between the treated and the control plants at Kombé ($_{\text{calculated}}t = 6.019$ with d.d.l. = 11), but not at Brazzaville ($_{\text{calculated}}t = 0.473$ with d.d.l. = 14).

After the first release, the evolution in the number of mealybug larvae and adults differs from the first week onwards for *E. flaviventris* and from the second week for *H. raynevali*. It is more difficult to attribute an influence to the second release, for this occurs when a significant difference has already been observed between the two groups of plants (fig. 3 A, B, C).

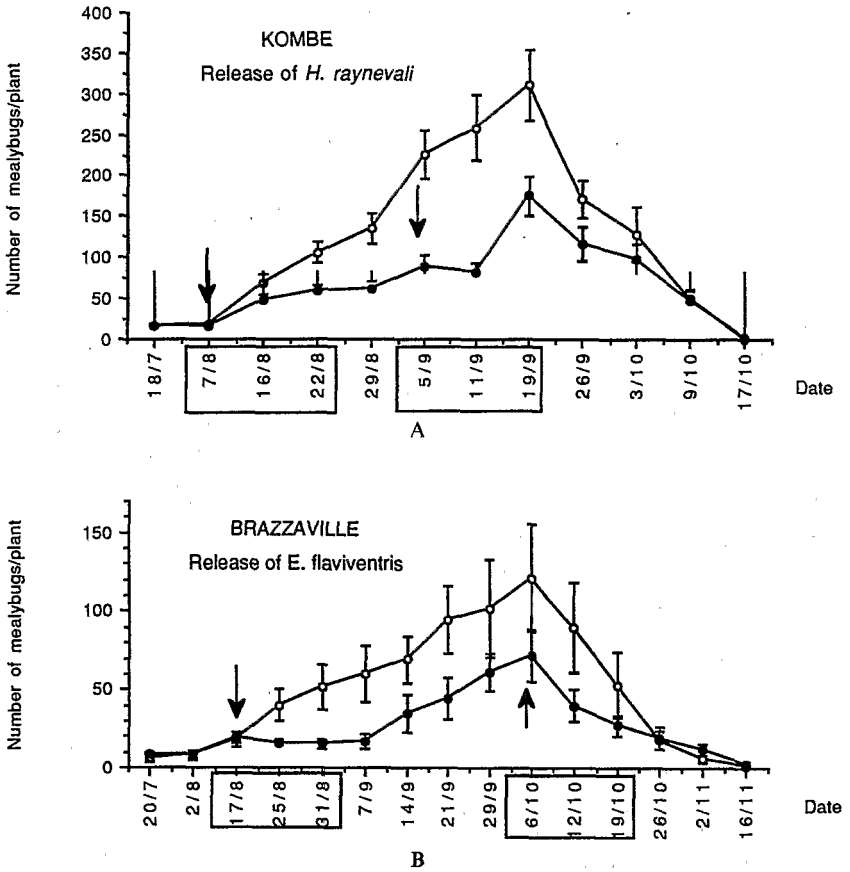

FIG. 1. — Evolution of the numbers of *P. manihoti* (all instars) on the stems of control (○) cassava and cassava treated (●) with 2nd instar larvae of *H. raynevali* (A) and *E. flaviventris* (B). Arrows indicate the biological treatments and the framed dates correspond to the activity period of coccinellid larvae. The standard deviation is given for each mean.

Effect of the introduction of coccinellid larvae on the three density levels of *P. manihoti*

The day of the first release, the great variability of the numbers of *P. manihoti* is illustrated by the extreme values ranging from 2 to 46 mealybugs/plant at Kombé and from 2 to 65 mealybugs/plant at Brazzaville in each treated group.

In order to assess more precisely the impact of the mealybug density on the predation of coccinellid larvae, the cassava plants of the treated and control groups are grouped in three classes, according to the densities of mealybugs they host, on the day of the first release (table IV). In each class the plants are chosen so that the numbers of *P. manihoti* in the control and treated groups of plants present no significant difference (1% level of significance) before the introduction of mealybug larvae (fig. 3 A to F).

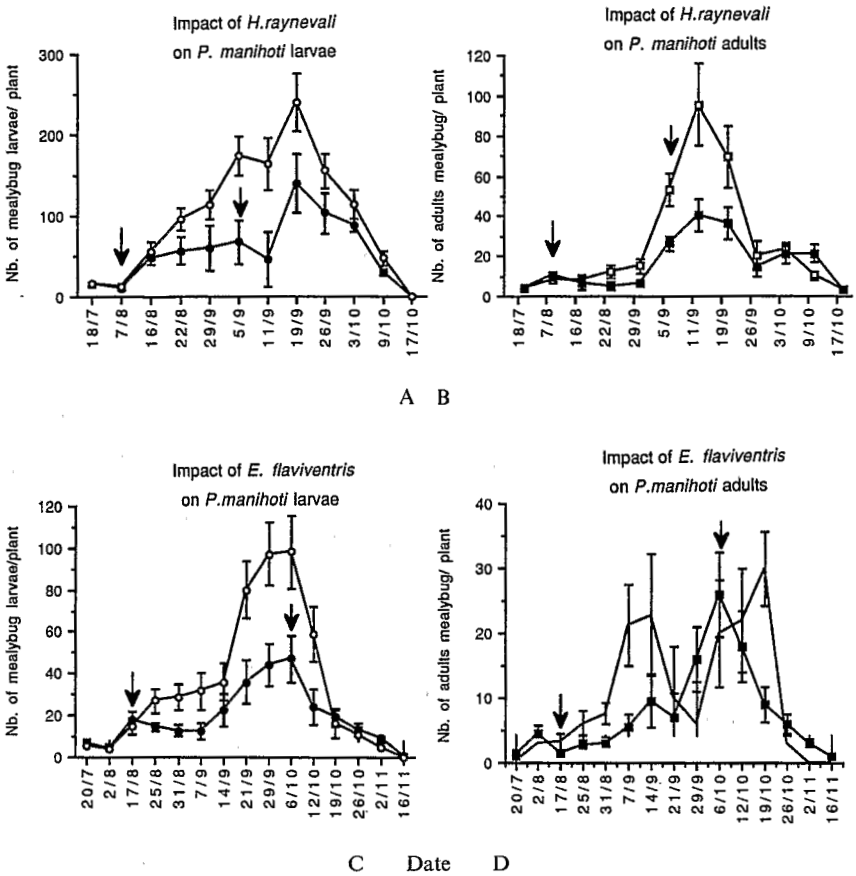

FIG. 2. – Evolution in the number of larval instars and adults of *P. manihoti* on the stems of control (○) cassava and cassava treated (●) with *H. raynevali* larvae (A and B) and *E. flaviventris* (C and D). The standard deviation is given for each mean. Arrows indicate the times of releases.

At Kombé, the presence of *H. raynevali* larvae at 7 ± 1 and 14 ± 2 mealybugs/plant slows down the expansion of the *P. manihoti* colonies (fig. 3 A and B); after 4 weeks, the numbers of pests have multiplied by 6 and 5 respectively for the above-mentioned densities on the treated plants, whereas on the control plants, they have increased by a factor of 26 and 11. At the highest density (35 ± 5 mealybugs/plant), the *H. raynevali* larvae manage to stabilise the numbers of mealybugs, whereas on the control plants the latter multiply by 14 (fig. 3 C).

At Brazzaville, the *E. flaviventris* larvae stabilise the *P. manihoti* colonies for 4 weeks, at densities on the day of treatment of 5 ± 2 and 18 ± 2 mealybugs/plant (fig. 3 D and E). At a higher density, they reduce the number of the pests from 44 ± 21 mealybugs/plant to 2 ± 2 mealybugs/plant in 4 weeks (fig. 3 F).

DISCUSSION AND CONCLUSION

The introduction of *E. flaviventris* and *H. raynevali* mealybug larvae had a significant impact on *P. manihoti* colonies. At the first release, the level of the

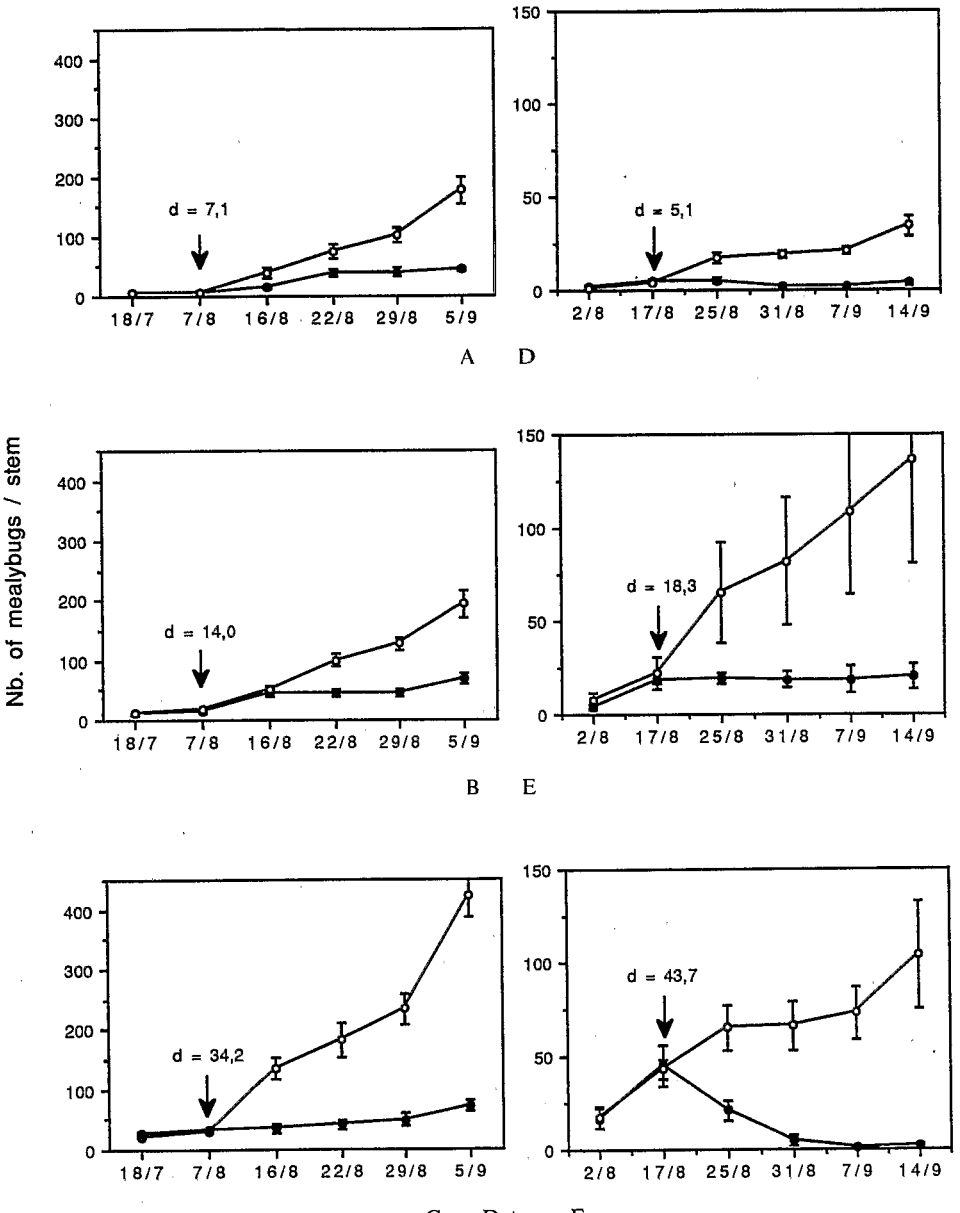

FIG. 3. – Evolution in the number of *P. manihoti* on the stems of control (○) cassava and the stems treated (●) with 2nd instar larvae of *H. raynevali* (Fig. 3, A, B, C) and *E. flaviventris* (Fig. 3, D, E, F) at three population densities, defined by the mean number of mealybugs per cassava plant (*d*) on the day of the biological treatment. The standard deviation is given for each mean.

TABLE IV. – *Density of mealybugs on the day of the 1st release. Each class is defined by the mean number of mealybugs/plant, together with the confidence margin at 5%, the extremes (–) and the number (n) of plants/class.*

	Kombe			Brazzaville		
	Class 1 $n=7$	Class 2 $n=7$	Class 3 $n=7$	Class 1 $n=7$	Class 2 $n=6$	Class 3 $n=5$
Control plants	7±2 (2-10)	14±2 (10-23)	31±3 (27-38)	5±1 (2-6)	22±3 (16-28)	43±11 (30-56)
Treated plants	7±1 (4-9)	14±2 (10-19)	34±5 (24-46)	5±2 (2-9)	18±2 (15-22)	44±21 (24-65)

impact was affected by the differences observed between the numbers of mealybugs in the control group and those in the treated group. It can be attributed to the released larvae as, at the time, the influence of local natural enemies was negligible.

The second release of coccinellids was carried out in the presence of many local *E. flaviventris*. At Brazzaville in particular, it becomes impossible to distinguish the impact of released coccinellid larvae from that of local predators and other natural regulation factors (rain, physiological state of the host-plant: LE RÜ *et al.*, 1991). AS FRAZER & GILBERT (1970) and HODEK *et al.* (1972) point out, one of the main difficulties encountered when quantifying predatory efficiency in outdoor conditions is to dissociate the impact of the predators studied from that of other natural regulation factors.

The introduction of *E. flaviventris* larvae maintained the numerical level of the pest below 20 mealybugs/plant for 3 weeks, whereas that of *H. raynevali* merely slows down expansion. For a comparable mean density of mealybugs in both localities (19±7 and 17±4 mealybugs/plant), the pest colonies consist of 91.3% of the larvae at Brazzaville and 41% at Kombé, on the day of the first release. Thus the introduction of coccinellid larvae took place 1 week before a sudden increase in the numbers of *P. manihoti* due to massive hatching. In those conditions, *H. raynevali* slowed down the expansion of the pest, but did not stabilise it. *E. flaviventris* acted on a population of larvae in the "aging" stage during which numbers increase less quickly. Thus the differences observed between Brazzaville and Kombé do not appear to be linked to the regulatory potentialities of the two species of coccinellids alone, but also to the age structure of the *P. manihoti* colonies.

During the first release, predation was exerted by coccinellid larvae on all the classes studied, although more intensively when the colonies increase in density. Thus the search for prey would be less efficient at low densities, owing to wider dispersal of mealybug on the plant, as FIREMPONG & KUMAR (1975) and OFUYA (1986) have shown with aphidophagous coccinellids. From the point of view of biological control, it would be preferable to treat the densities of the pest prevalent at the beginning of infestation, when their number is around 10 mealybugs/plant and they have little effect on the losses of tuber crops (NEUENSCHWANDER *et al.*, 1989). A smaller number of coccinellid larvae will be needed.

The variable study conditions (edaphic factors, plant material, microclimate factors, associate entomofauna) are the source of differences between the two plots of crop land in the abundance and in the age structure of *P. manihoti*. For this

reason, no strict comparison between the predatory potentialities of the two coccinellid species could be established. Similar experimentation conducted on a single test plot of land (standard experimental conditions) would enable a more accurate comparison of these two predators.

The impact of predation by coccinellid larvae depends on the density of the pest colonies, but also on their age structure. This result agrees with observations made by FRAZER & GILBERT (1976) who showed that the impact of coccinellids associated with *Acyrthosiphon pisum* Harris (Hom. Aphididae) cannot be assessed in relation to the prey/predator density variations alone, but that the age structure of the pest colonies must also be taken into account, as well as the voracity of the predator and temperature. As these different parameters cannot be controlled simultaneously in outdoor conditions, we propose to study them later in laboratory conditions.

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