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Investigate factors that affect hosts suitability and hosts range of Tephritidae: Case of tomato fruit fly *Dacus punctatifrons* Karsch, Bambili, Cameroon

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Abstract
Factors that influence development and host selection by pest are important for pest suppression in agro-ecology. Fresh eggs of *D. punctatifrons* were collected and inoculated to six local fruits in a non-choice test; developmental parameters of larvae were compared. Tomato, water-melon and sweet-pepper were revealed as preferential host of *D. punctatifrons* (complete development in 31.24 days). Larvae rearing on Guava and mango completed their life-cycle in a relatively longer time (38.63 days), those rearing on orange, all died at stage (L_1). Principal Component Analysis showed that feeding significantly affect life traits and demographic parameters of *D. punctatifrons*. Larval suitability compare to existing databases reveals that, hosts of *D. punctatifrons* in the laboratory and in nature were different. This could means nutritional criterion is not the only determinant of host selection by *D. punctatifrons* in nature. Ecological factors (competition and farming practices) may influence more.

Keywords: *D. punctatifrons*, treatments, life-traits, host-selection, agro-ecology

1. Introduction

In Africa, the genus *Dacus* is represented by 177 described species [1] and a further 25 undescribed taxa (White, personal communication). It includes major pests such as *Dacus ciliatus*, *Dacus vertebratus* and *Dacus punctatifrons*. In Cameroon the loss of tomato harvest is caused by a fruit fly *D. punctatifrons* Karsch (Diptera: Tephritidae) and two caterpillars firstly *Helicoverpa armigera* and more recently tomato miner *Tuta absoluta*. Among them, *D. punctatifrons* is the most notorious pest, responsible for more than 80% of the total yield losses of tomato fruits [2]. *D. punctatifrons* is multivoltine, olygophagious and endemic pest to central Africa [3-4]. This limited geographical distribution may explain why only few studies are found in the literature.

Although several samplings and laboratories experiments have been done for various fruit fly species performance in relation to their host plant such as the Mexican fruit flies *Anastrepha ludens* [5], *Bactrocera tryoni* [6] and Mediterranean fruit flies *C. capitata* [7-8] only few studies have been carried out in looking the effect of the host plants on biological traits of *D. punctatifrons*. *D. punctatifrons* is well known in Cameroon and, it was pointed out for the first time as to cause enormous losses of marketable tomato in the center region [9]. Some others contributions were done on the biology, ecology and even the impact of the pests on the yield of tomato [10-14]. A recent contribution of [15] on the monitoring of the activities of this pest has confirmed that, in the natural context, the host range of *D. punctatifrons* was limited to some cucurbits and tomato [16].

Following the hypothesis of evolution concerning insect-plant relationship, a female fly will choose plant species that maximize larvae survival and development [17-18]. According to this preference-performance theory, females preferred appropriate resources for their offspring, the quality of nutrient contained in the food seem to be the criteria of host selection in the nature. [8] noticed that in phytophageous insects, the growth rate, longevity and reproductive success are cumulatively determined by a number of critical factors experienced by the individual during larval development stage. According to [19] polyphagous insects select their plant hosts based on nutritional values.

Because, *D. punctatifrons* in the nature is known as tomato's and cucurbits pest, the question was can these two hosts be the only ones which possess quality nutrients for the development of that pest? Or can *D. punctatifrons* develop and complete their life cycle on others potential hosts (host not yet infested in the nature) found in the surrounding environment? We therefore hypothesized that the nutritional determinant must not be the only discriminative factor that guides this pest on the choice of their host in the nature.
The aim of this work was to test the suitability of some fruits on the life cycle of the *D. punctatifrons* in a pest-host relationship in the laboratory, to attempt to highlight some determinants factors that influence host selection by *D. punctatifrons* in the nature.

2. Materials and Methods

2.1. Laboratory experiment

From March to July 2019, eggs of *D. punctatifrons* were extracted on fresh infested tomato harvested in an experimental garden. In the laboratory tomatoes were simply dissected and the fresh eggs of Dacus carefully extracted. These eggs were then inoculated on six different fruits, and incubated in insectariums. They developments rate were compared using Kruskal-wallis test. The tested fruits were tomato *Lycopersicon esculentum* Miller (Solanaceae) variety Rio-Grande, water melon *Citrullus colocynthis L.* (Cucurbitaceae), guava *Psidium guajava L.* (Myrtaceae), sweet pepper *Capsicum annuum L.* (Solanaceae) mangoes *Mangifera indica L.* (Anacardiaceae) and orange *Citrus sinensis L.* (Rutaceae) harvested from gardens within the study area. The experiment were conducted under the temperature 76.9°F (19.5 ± 2 °C), and at mean relative humidity 57± 8%.

2.2. Experimental design

A total of 240 eggs were treated with different food, this food were made up 6 local fruits: tomato (as the control treatment) (T0), water melon (T1), sweet pepper (T2), guava (T3), orange (T4) and mango (T5). The developments of 40 eggs were individually followed up from eggs to dead on each treatment. In the laboratory, the fruit were slice in to pieces and put in 24 Petri dishes. That is 10 eggs were inoculated to each Petri dish, these eggs were observed daily and their hatching dates were recorded. The interval between the oviposition and hatching dates were considered as the incubation period. Hatching larvae were observed daily and food substrates were also changed daily. These observations allowed us to determined three distinctive stages of larval developmental periods. The first is the molt of larval stage1 (L1) to larval stage2 (L2), second is molt of larval stage 2 to larval stage 3 (L3), and the third is the transformation of (L3) to pupae. Unlike (L1) and (L2) which are whitish in color, (L3) is yellowish in color. The evaluation of the interval of time which separates two consecutive molts permitted us to determine larval developmental duration on it specific host fruits. The time interval between the dates of hatching of L1 larvae to the pupation were considered as the larvae duration, and the time interval between pupation to imago were considered as pupae duration.

2.3 Adults lifespan

Immediately after emerged adults flies were placed into different insectariums, and were feed with the juice extract of the fruit where they eggs hatch and develop for the 6 different fruits. The juices were changed twice a day while daily; observations were carried out to record mortalities of both sexes by simple counting. The times interval between the date of emergence and the death of each individual was considered as the adult longevity.

* Emergence percentage: $\%E = \frac{NIE}{NP} \times 100$

%E: percentage of emergence;
NIE: number of individual emerged;
NP: number of pupae.

*Sex-ratio:
Adults that emerge were identified according to their sex; using this formula:

$$SR = \frac{NM}{NF}$$

SR: Sex-ratio;
NM: number of male;
F: number of female.

*Larval viability:

$$ILS = \frac{NE}{NP}$$

ILS: Index of larval survival;
NE: number of egg;
NP: number of pupae.

2.4 Statistical analyses

Statistical analysis were conducted using Kruskal-wallis test, Shapiro-wilk test showed that the distribution of data was not normal. The developmental duration, pupal and adult weights as well as adult longevity of both sexes were compared using Kruskal-wallis test. Principal Component Analysis (PCA) was also used to discriminate the life trait parameters which are being influenced by the feeding treatment, PCA also helps to gather fruit base on the similarity of their effect on the biology of the insects. The analyses were realized using the statistical software XL STAT version 7.5.2.

3. Results

3.1 Effect of the food types on the biological cycle of *Dacus punctatifrons*

The incubation, duration of eggs, the larval developmental duration, the pupal duration and even the lifespan of *D. punctatifrons* adults are influenced by the food treatments, analysis revealed high significant variation: N=240, DF=4, *P*<0.001. On the 6 treatments, only orange did not allow the development of the fly. On more than 40 larvae subjected to this treatment, none of them reached the L_2 stage (Table1).
The durations of both eggs incubation and the passage of the larvae from stages 3 to pupa where invariable whatever the food treatment (N=240, DF=5, *P*>0.05). On the other hand the food treatment influences significantly larval development duration (N=240, DF=4, *P*<0.001), this duration varies very significantly particularly between the passage of the L_2 to L_3 larva and range between 7.22±0.41 to 14.4 ±3.98 days. The table 1 also showed that the treatment significantly influenced total development duration (N=240, DF=5, *P*<0.001). This duration is shorter on the tomato 31.24 ±4.38 days and longer

on mango 38.63±4.68 days. The result also showed that, the food treatment influenced significantly the longevity (N=240, DF=5, *P*<0.0001). Adult longevity ranged between (11.47±2.52 - 18.19±2.53) days for the insect feeding on goyava and on tomato respectively.

Table 1: Effect of the food treatments on the development duration of *Dacus punctatifrons* in days at an average temperature of 19.5±0.2 °C

Treatment	Incubation	L_1-L_2	L_2-L_3	L_3-pupea	pupae-adult	Total duration	Adult longevity
Watermelon (T_1)	2	5.22±0.41	7.22±0.41	3	14.8±0.88	32.24±1.71	16.8±3.29
Mango (T_3)	2	5±0.0	14.4±3.98	3	14.23±0.69	38.63±4.68	13.2±2.26
Guava (T_4)	2	5.31±0.41	7.63±0.94	3	14.23±0.75	32.18±2.16	11.47±2.52
Sweet pepper (T_5)	2	5±0.0	8.91±0.41	3	14.04±1.81	32.95±2.2	16.97±2.77
Tomato (T_0)	2	5.33±0.47	7.33±0.47	3	13.33±3.43	31.24±4.38	18.19±2.53
Orange (T_2)	2	5±0.0	0	0	0	0	0
Note T= treatment ; L1=Larvea stage 1 ; L2=Larvea stage 2; L3=Larvea stage 3							

3.2 Graphic representation of the effect of treatments on the development of *Dacus punctatifrons*

Principal Component Analysis (PCA) shows the action of each treatment on larva's development and also the similarities between the treatments, only components 1 and 2 were taken into account, components 3 and 4 neglected then their eigenvalue is much lower than the threshold 16.30 which is the value from which one can consider that a component has importance. The first component gave majority of the information (68.34%), the second gave a little information (20.23%). Thus three distinct groups were formed on various areas of the graph. The first made up of tomato, water melon, and sweet pepper, the second; mango and guava finally a third made up of orange only. The first group showed that these three treatments: watermelon, sweet pepper and tomato significantly influenced the parameters such as the longevity and the duration from pupa to adult. The second group made up of mango and guava influenced the passage of the phase L_2 - L_3 and the total developmental duration from larvae to adult. The third group which is orange does not allow the development of the insect; all the larvae died after hatching at stage larvae L_1. Exploitation of this graph also indicated that watermelon and the sweet pepper which showed the same effects on the growth of the larvae can be used as substitution plants in the case of absence of the tomato which is the principal host of *D. punctatifrons* considered in this work as the control plant host. The second group showed that the larvae of *D. punctatifrons* can also develop on mango and on guava, but this could be possible only in the extreme cases of absence of the hosts of the up cited host plants. Then the longer duration of total development proves the difficulty of these larvae to develop on the plants of group 2. Finally a third group formed by orange which simply stopped the development of *D. punctatifrons* at the stage of larvae L_1.

The PCA also showed that, insect's longevity, development duration from L_2-L_3, pupa-adult and duration of total development are the traits of life which are influenced by the food treatment. On the other hand the parameters like the incubation duration, the pupae duration whose arrows remained around the center of symmetry are not influenced by the type of treatment (Fig 1).

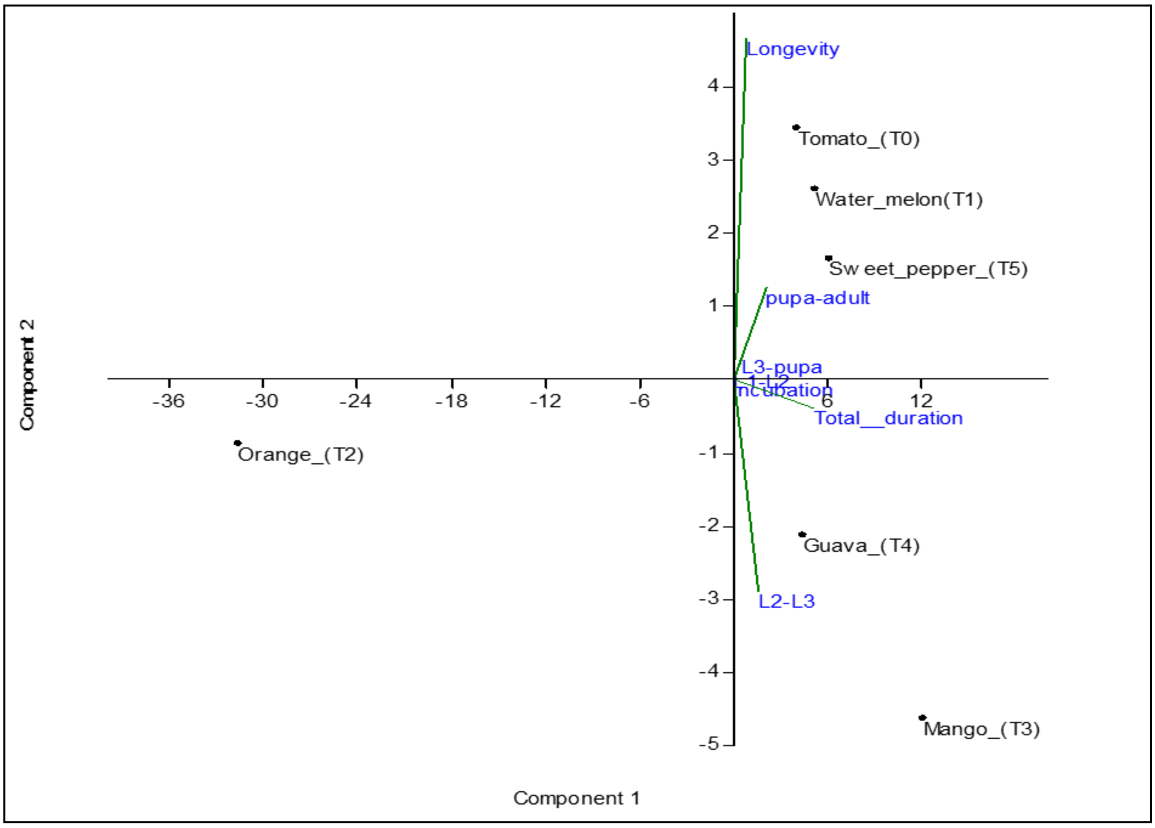

Fig 1: Principal Component Analyze (PCA) showing the three groups of food substrates being formed on the basis of similarity of their effects on the biological parameters of *Dacus punctatifrons*.

3.3 Effect of the food types on hatching and emergence percentage of *Dacus punctatifrons*

There was no significant difference among eggs hatched percentage on different treatment. Thus, the hatching percentage was: (84%) for sweet pepper, (68%) for tomato, (75%) for watermelon, (70%) for mango and (62%) for guava. For all the larvae that reach the stage of pupae, 100% of adults emerged on all treatments without any difference (Table 2).

Table 2: Hatching and emergence percentage of *D. punctatifrons* on different treatments

Treatments	n° of eggs	n°of eggs hatch	%hatching	n°of pupae	n°of adult emerge	%emergence
Sweet pepper	80	67	84	67	67	100
Tomato	60	41	68	41	41	100
Water melon	60	45	75	45	45	100
Mango	60	42	70	42	42	100
Guava	60	37	62	37	37	100

Note n°= number

3.4 Effect of treatments on the sex-ratio

Females are distinguished from the males by the development of a long ovipositor from 2 to approximately 3 cm at their abdominal extremities. At the emergence in the laboratory, independently to the type of treatment, the sex-ratio was “female biased”. The highest female number was obtained on tomato (75.6%) and lowest on guava (54%) (Table 3).

Table 3: Number of adults and repartition of sex of *Dacus punctatifrons* according to feeding treatment

Treatments	Male (M)	Female (F)	M+F	S/R=M/F	Percentage of female (%)
Sweet pepper	25	42	67	0.55	62.69
Watermelon	13	32	45	0.40	71
Tomato	10	31	41	0.32	75.6
Mango	18	42	60	0.42	70
Guava	17	20	37	0.65	54

Note M= male ; F=femelle

4. Discussion

4.1 Effect of treatments on larval developmental duration, pupa duration and adult longevity

The incubation duration and the duration from L_3 larvae to pupa were not related to treatments; this can be due to the fact that at these periods of their live cycle, neither egg, nor L_3 larvae ingest food. So this phase was control only by the environmental conditions like the temperature and weather [20]. The larvae developmental periods were significantly different between the treatment this may be due to the different nutrient content in the fruits and the quantity of water they contained. Total developmental cycle of *D. punctatifrons* from eggs to the adults is shorter on the tomato fruits follow by sweet pepper, watermelon, larvae that feed on mango has the longest developmental duration the range was (31-38) days. The faster the development of the larvae occur, the better is the fitness of the larvae which are less exposed to the possibility of being attacked by a predator or by the parasitoids which are more actives during larval stages of the insect. [21] pointed out the amount of protein in the diet to influences more the development of immature individuals. Surprisingly, the quantity of protein contained in 100g of tomato was 0.7g far lower to the one contained in 100g of guava 2.55g and at contrary, the quantity of water 93.1g/ 100g of tomato more abundant than Guava with 77g/100g of guava. Water food may be easy to extract, to ingest and to digest than solid food. [22] have demonstrated that, adult fruit flies require water every few days for survival, and frequently they have been observed imbibing dew or rain droplets, knowing that in this experiment, water was not provided to insect, all the water needed for their development came from the food provided to them; choice of liquid foods is certainly dominated by the need for water rather than presence of essential proteins. [23] observed the total developmental cycle of *D. punctatifrons* from eggs to the adults within 25.95 days that is different from the means of 31.24 obtained in our experiment on the same tomato substrate. This difference may be due to temperature which was 19.5±0.2 °C in the present study and 20±0.2°C in these authors condition. Apart from the host fruit quality, temperature may also be responsible for the variation in development duration. [24] emphasized on the fact that small change in temperature can have a great acceleration on metabolite processes.

Longevity of adult differed significantly between the 6 treatments. The longest adult lifespan was found on insect feeding on tomato and the shortest on guava. Once again, tomato appears as the best fruit in terms of nutrient proportion for adult longevity. The mean longevity of flies feeding on tomato was 18.19 days superior to the longevity obtained by Okolle and Ntonifor (2005) [23] that was maximum 16.85 days on female of *D. punctatifrons* feeding with a mixture of tomato juice, sugar and honey. Additional sources of protein and sugar added by these authors to the larvae feeding in their experiment did not increase the longevity of this insect. No difference was observed between longevity of male and female. Guava, with 2.55g protein containing in 100g is, the highest protein fruit amount tested treatments, but adult rearing on guava has minor longevity duration than those rearing on tomato that has just a small quantity of protein (0.7g/100g), so, the quantity of protein contained in the fruit didn’t influence the longevity of insect positively, but only the quantity of protein that could be extracted from the food by adult insects influenced. So water seems to facilitate nutrient taking by this fly which has a spongy mouthpart (labella). [22] reported water to be very important components in flies’ development, their experiments reviewed that fruit flies without water succumb within 3 days. When provided water they lived about twice as long.

Also some components of the fruit can contribute to stop the development of the larvae. [25] noted that some plant groups possessed apparently chemical barriers to potential herbivorous insects, and restrict accessibility to relatively few insect. Insects that infested such plants probably pre-adapted to the use of chemical produced by the host plants. [26] noted that in the farm conditions, secondary metabolite produced by plants affect negatively the development duration and pupation of *Ceratitis capitata*. Certainly oligophagous insects like *D. punctatifrons* do not have substances that are able to digest such substances.

4.2 Effect of treatments on emergence percentage and sex-ratio

The survivorship of eggs and the emergence of adults from the pupae do not depend on the fruit quality; since they don't consume the fruits, eggs and larvae require only weather factors like humidity and temperature for their emergence. All the larvae that reached the pupae stage emerge in to adult in all the treatment.

The sex-ratio at emergence is female biased. Tomato is once again, the best treatment to *D. punctatifrons.* Tomato contributes more to the success of this fly by producing the highest number of female. This can be an advantage in the life history of this insect because generally, animal populations where females are many than males developed better because competition for mating is reduced. Unfortunately, this capacity of producing many females contribute to increase the harmful effect of this pest because only the female flies are directly threatened tomato fruit. [27] has shown that the sex-ratio of some Tephritid including *Dacus cilliatus* pest of cucurbit in Reunion Island vary according to Tephritid group and according to their host plants.

4.3 Comparison of *D. punctatifrons*/hosts relationships in the laboratory and in the natural context

Although *D. punctatifrons* were able to complete their development cycle on 5 of the 6 tested host in laboratory, this was not the reality in the natural context. In the study locality where these plants were present only two (tomato and water melon) of the tested hosts were chosen by female *D. punctatifrons* to lay their eggs. The work of [28] showed that females of *C. capitata* did not lay they eggs preferentially on the most nutritive parts of the fruits. Moreover in Brazil *C. capitata* is known as pest of pawpaw, in Mediterranean zone it is devastating apple fruits, in Central Europe it is the pest of citrus [29] and in Cameroon, it is the main pest of pepper [30]. Other examples of switching of host in our study area are from [31-12], they notice that Guava and Mangoes are ecological niches that are occupied by *Bactrocera invadens* which is a recent invasive pest and that may be more competitive than *D. punctatifrons* the native species. In an evolutionary approach *D. punctatifrons* is the oldest fruit pests in the area and may have been force to switch from mango by invasion of *Bactrocera invadens.* This assertion can be reinforcing by the finding of [12] that showed that in Ngaoundere although the two pests were still using mango as host, *Bactrocera invadens* were dominant with (98.5%) and *D. punctatifrons* only (1.5%) of the flies population. Previous study showed that females of the flies were able to choose only two families of plants that it Solanaceae (tomato) and some Curcubitaceae to lay their eggs in the natural context [32-33-15]. These finding are not matching with a result obtained from a non choice experiment in the laboratory that showed that eggs of *D. punctatifrons* inoculated to host substrates, can develop and complete their cycle on sweet pepper, guava, and mangoes. So the quality of the food is not a limiting factor for pests' development. The nutritional values of tomato, watermelon and sweet pepper are similar in term of water, vitamins and nutrients contents but what can explain the fact that in the study area, *D. punctatifrons* attacks only tomato? These observations reinforce our analysis on the fact that the nutritional determinant is not the only factor that can guide the pest to select their host. The fact that in the laboratory the larvae of *D. punctatifrons* were able to develop on these two fruits showed that sweet pepper could have been a natural host of *D. punctatifrons* if *C. capitata,* its instant competitor wasn't the pest of sweet pepper.

Another explanation could be the cultural practices of the locality, which may influence the pest in selecting their plant's host in the nature. From the life history of many fruit flies, it was observed that the food preferences vary in time and space, this confirm the analysis according to which the choice of the host by a pest should not be more of the result of the trophic interactions between the insects and plants but more of competition amount the insects species present in the locality. The presence of a pest in a particular host may have excluded the other species from colonising the same host in the area. [34] also think that insects/plants associations are control by the ecological processes.

Another explanation could be that, some host plant in the natural context produce secondary metabolites that stimulate the production of pheromones that can attract some insects or repel them by reducing their appetite.[35] quoted by [26] showed that certain plants hosts often contain the toxic secondary metabolites which could either directly stop or slow down the development of the larvae in the absence of mechanisms of tolerances, or indirectly by increasing the performance of the carnivorous insects by providing for example chemical refuges or substances such as the pheromones [36].

5. Conclusion

The quality of nutrient significantly affected some life trait parameters of *D. punctatifrons* such as developmental duration, pupae duration, the duration from egg to adult and the longevity of adults. Tomato appears to be the best host of *D. punctatifrons*.

The relationship between *D. punctatifrons* and the host plants is not a permanent relation; it is a dynamic relation that is structured by competition that exists within fruit-eating pests, farming practices, and some chemical substances produce by the plant host.

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