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A COMPARATIVE STUDY OF SEVERAL STRAINS OF THE *TETRANYCHUS NEOCALEDONICUS* COMPLEX AND STERILIZATION TESTS OF MALES BY X-RAYS

BY

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Five populations of *Tetranychus neocaledonicus*, collected in various biotopes from different plants, were studied in order to become informed about variation in this species. It appeared that all strains differed with respect to longevity and fecundity. Crossings resulted in incompatibility, hybrid inviability, hybrid sterility and hybrid breakdown. It is suggested that the great interpopulational variation as found in *T. neocaledonicus* is a consequence of the haplo-diploid system.

An important difference in longevity and fecundity between mated and unmated females has been stated for all strains. Outcrosses with males from another species indicated that it is not the fertilization, but the insemination only which induces the enhanced fecundity in *T. neocaledonicus*. Irradiated males appeared to be able to inseminate females effectively, resulting in the prevention of viable diploid offspring.

The spider mite *Tetranychus neocaledonicus* André is a species of considerable economic importance on all kinds of crops, and has a wide distribution in the intertropical zone. It resembles in many respects the polyphagous spider mite *Tetranychus urticae* Koch, which has also a wide range of distribution but predominantly in the temperate zones.

In *T. urticae* a great variation is known, characterized by different degrees of genetic affinities between populations (Helle & Pieterse, 1965). The conspicuous differences in colour between populations seem also to reflect the existing interpopulational variation. The colour pattern of living mites has often been used as a criterion to distinguish between certain ecotypes or even species within the *T. urticae* complex (Davis, 1952; Keh, 1952; Boudreaux, 1956; Ghobrial *et al.*, 1969). However, specimens of morphologically identical populations may be reproductively isolated from each other, whereas sometimes populations characterized by different body colours show good genetic affinity to each other. Owing to the similarities between *T. urticae* and *T. neocaledonicus* one would expect also a large degree of interpopulational variation in *T. neocaledonicus*. However, within the latter species there seems lesser variation in the morphological characteristics. Specimens from New Caledonia (P. Cochereau, Coll.), Dahomey (IRCT, Coll.), Louisiana, USA (H.B. Boudreaux, Coll.), Madagascar, Réunion, Mauritius and the Comores have been examined but no clear morphological differences were found. Also there appeared no sufficiently clear difference in body colour between

a number of strains that were examined originating from Madagascar and the USA. In order to obtain information on the genetic variability of *T. neocaledonicus* some physiological aspects of various strains were studied together with reproductive affinities. The physiological aspects concerned longevity and daily egg production.

As differences in longevity and daily egg production between mated and unmated females were found, attention will be paid to this phenomenon in the final part of this paper.

LONGEVITY AND FECUNDITY COMPARED IN DIFFERENT STRAINS

Material and methods

Four *T. neocaledonicus* strains came from Madagascar and a fifth one from the USA :

Tuléar strain, collected in Tuléar (altitude 10 m) from *Gossypium hirsutum* L. (Malvaceae); Ihosy strain, collected in Ihosy (alt. 750 m) also from *Gossypium hirsutum*; Tananarive Ipomoea strain collected in Tananarive (alt. 1250 m) from *Ipomoea* sp. (Convolvulaceae); Tananarive Sechium strain collected in Tananarive (alt. 1250 m) from *Sechium edule* L. (Cucurbitaceae); Louisiana strain obtained from Baton Rouge, Louisiana, USA, where it had been reared on *Phaseolus* sp. during several years. All strains were reared on the terminal leaves of cotton plants using the plexiglass cell technique (Gutierrez, 1967). Cotton plants were grown in a glasshouse by hydroponic culture (Braud, 1967). Cotton was chosen as a host plant as it is readily accepted by all strains. The cotton leaves were renewed each week. The plexiglass cells were placed in sealed glass chambers, in which a potassium carbonate solution maintained 50% relative humidity. The glass chambers were placed in an incubator at 25°C, with a photoperiod of 12 hours daily. From previous experiments it was learned that these thermic and hygrometic conditions were optimal for the Ihosy strain.

Approximately 100 female teleiochrysalids (R_3) from each strain were divided into two equal groups, one for the study of mated females and the other one for the study of unmated females.

Females to be mated were placed separately in cells, each female together with an adult male. In case of loss, the male was replaced during the experiment. The virgin females were all kept isolated during their life time. Observations and counts of mites and eggs were made every day.

Results and discussion

Table I shows the mean longevity (number of days survived by 50% of the females) and the mean egg production per female of each strain. The net reproduction rate of each strain, assuming the percentage of females in the progeny to be 100%, can be derived from the mean egg production per mated female. Figs 1 and 2 represent the daily egg production of unmated and mated females, respectively, of the Madagascar strains, during life time.

TABLE I

Survival and fecundity of mated and unmated females of five T. neocaledonicus *strains*

Strains	Mated females				Unmated females			
	No of ♀	Mean longevity in days	Maximal longevity in days	Mean egg production per ♀	No of ♀	Mean longevity in days	Maximal longevity in days	Mean egg production per ♀
Ihosy	55	13	29	80.3	53	28	43	53.3
Tuléar	36	12	19	60.0	35	18	33	53.1
Ta. Ipom.	52	14	23	62.9	55	28	41	32.3
Ta. Sech.	36	11	19	44.7	38	14	24	25.6
Louisiana	49	13	26	59.3	55	28	45	42.8

The following conclusions can be made :

1. In all strains studied, mated females laid more eggs and lived shorter than unmated females. This phenomenon has also been reported for another species of the intertropical zone, viz. *Tetranychus desertorum* Banks (Nickel, 1960). *T. urticae* from the temperate zones does not exhibit this feature.
2. The strains Ihosy and Tulear taken from cotton plants had a higher net reproduction rate than the other strains studied. This was not unexpected as we chose the experimental conditions optimum for the Ihosy strain.
3. The unmated females from Tulear laid nearly twice as many eggs at the beginning of their life, as compared to the females of the Ihosy and Tananarive *Sechium* strains. The Tananarive *Ipomoea* strain produced still fewer eggs.

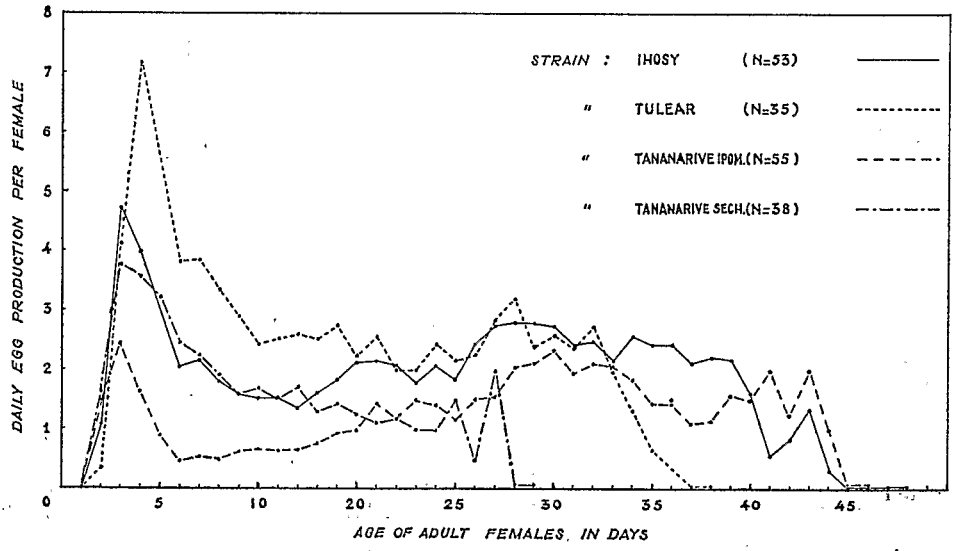

Fig. 1. Daily egg production of unmated females of four *T. neocaledonicus* strains reared under constant conditions of temperature and humidity (25° and 50% R.H.).

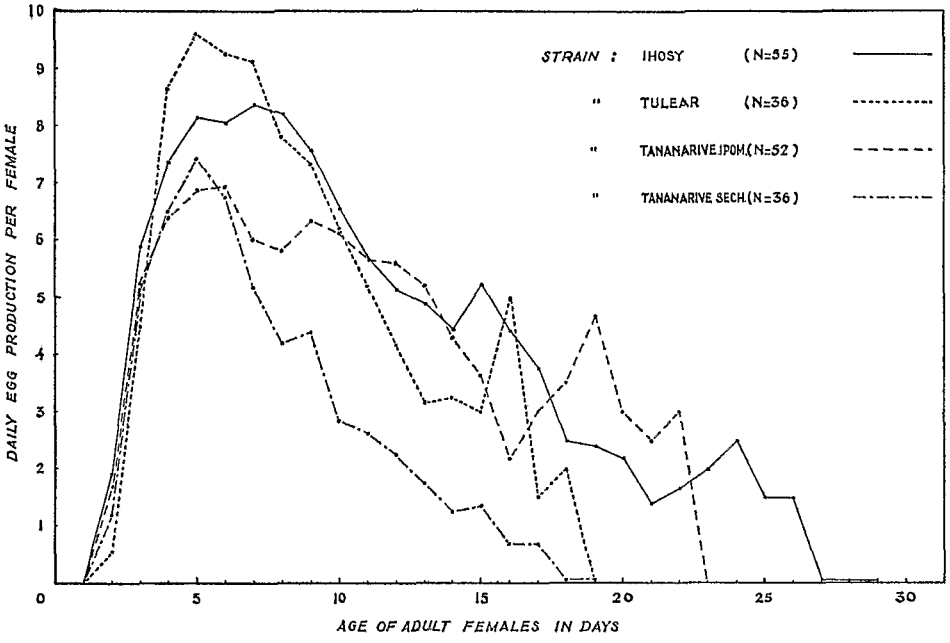

Fig. 2. Daily egg production of mated females of four *T. neocaledonicus* strains reared under constant conditions of temperature and humidity (25° and 50% R.H.).

4. A comparison of longevity between the strains was based on the number of days before 50% of the females died. For mated females this varied little from one strain to another (11 to 14 days); for unmated females, however, the differences were greater (28 days for Ihosy, Louisiana and Tananarive *Ipomoea*, 18 days for Tulear, 14 days for Tananarive Sechium).

To make more precise comparisons between the total egg production of different strains, the sources of variation based on the observed differences were analysed (cf. Table II). The two major factors involved were "host plant" and "mating". The "host plant" factor was divided into two categories according to whether the strain originated from cotton or from another plant; the strains from

TABLE II

Comparison of the fertility of five T. neocaledonicus *strains*

Host plant	Strains	Mating			
		Mated ♀		Unmated ♀	
		♀	eggs	♀	eggs
Cotton	Ihosy	55	4 417	53	2 826
	Tulear	36	2 449	35	1 859
Other than cotton	Louisiana	49	2 907	55	2 356
	Tana. Ipom.	52	3 269	55	1 775
	Tana. Sech.	36	1 608	38	971

cotton were subdivided into those from Ihosy and Tulear; strains from other plants were considered individually. These distinctions allowed us to make an analysis of variance of egg production for the five strains.

It appeared that there was a significant difference ($p < 0.01$) for all strains in egg production of mated and unmated females. With respect to the interstrain variation in egg production, it was found that the cotton-strains (Ihosy and Tulear) differed significantly from the other strains ($p < 0.01$). From a complementary F-test it appeared that the differences in egg production between Ihosy and Tulear were also significant.

GENETIC COMPATIBILITIES BETWEEN DIFFERENT STRAINS

Reciprocal crosses were made between a number of *T. neocaledonicus* strains in order to examine the degree of their reproductive isolation. In addition, a reciprocal cross between one of these strains and a strain of *T. urticae* was made. The results of outcrossing experiments were compared with corresponding data of the parental strains.

Material and methods

The following strains were used: Tulear strain; Ihosy strain; Tananarive Ipomoea strain; Tananarive Sechium strain (see above). Louisiana white eye strain (Lawe). This strain was derived from the Louisiana strain and is characterized by the absence of eye pigment under control of a single recessive gene. The use of this recessive marker permitted us to determine the sex of larvae in progenies obtained from outcrosses of Lawe females with wild-type males. A *T. urticae* strain, originally found on *Rosa* spec. in Tananarive, was also involved.

All strains were kept on cotton. The crossing experiments, involving both intra- and interstrain crosses, were carried out in plexiglass cells. The intrastrain crosses served as a reference for the interstrain crosses. Egg hatchability and total egg production were determined. For the production of F_1, F_2 etc., several unmated females of a certain strain were placed each in a plexiglass cell together with a male. They were transferred to new cells after the production of approximately 10 eggs. The percentage nonhatched was determined and the viable progeny was reared to adulthood. In a number of cells no males were added, in order to study the production of haploid offspring.

The total egg production of the females was determined by putting unmated females in plexiglass cells, either together with a male or not, and the daily egg production was scored. After counting, the eggs were destroyed and the females (and the males) remained in their cells.

Results and discussion

Curiously enough, the males seemed always attracted by the young females even in interspecific crosses. Attraction between males and females in interspecific

crosses is not always the rule and sometimes there is attraction in one direction only (Gutierrez & Helle, 1971).

Table III presents the results of intra- and interstrain crosses. It appeared that *T. urticae* and the Ihosy strain did not hybridize. The isolated position of Tananarive Ipomoea strain, characterized by incompatibilities in crossings with other strains, is even more remarkable, since it concerns an intra-specific situation. With respect to the other data it is apparent, that the highest mean egg production per female of each strain is always found when the females concerned were mated by males originating from the same strain.

TABLE III

Results of rearing and crosses

	Parents			F_1		
♀ × ♂	No of ♀	Mean longevity of ♀ in days	Mean egg production per ♀ ± t. S_m	♀ (%)	♂ (%)	Non-viable eggs (%)
Ihy × Ihy	55	13	80.3 ± 7.7	70.3	27.2	2.5
Ihy	53	28	53.3 ± 7.9	0.0	97.9	2.1
Ihy × Tul.	27	13	41.0 ± 4.6	62.2	32.3	5.5
Ihy × Ta. Ipo.	30	17	46.1 ± 6.5	0.4	97.2	2.4
Ihy × Ta. Sech.	18	13	46.6 ± 8.8	78.4	18.5	3.1
Ihy × La. we	18	13	69.7 ± 9.1	67.9	29.7	2.4
Ihy × T. urt.	22	22	45.3 ± 6.4	0.0	96.8	3.2
Tul. × Tul.	36	12	68.0 ± 7.4	75.1	21.6	3.3
Tul.	35	18	53.1 ± 6.7	0.0	97.1	2.9
Tul. × Ihy	34	10	35.4 ± 6.2	72.9	18.6	8.5
Ta. Ipo. × Ta. Ipo.	52	14	62.9 ± 7.6	84.7	12.6	2.7
Ta. Ipo.	55	28	32.3 ± 3.5	0.0	96.9	3.1
Ta. Ipo. × Ihy	34	26	28.9 ± 3.1	0.0	97.1	2.9
Ta. Ipo. × Ta. Sech.	24	23	30.0 ± 5.1	0.7	93.8	5.5
Ta. Ipo. × La. we	28	18	21.4 ± 3.3	0.0	95.7	4.3
Ta. Sech. × Ta. Sech.	36	11	44.7 ± 4.8	72.9	23.9	3.2
Ta. Sech.	38	14	25.6 ± 4.2	0.0	95.2	4.8
Ta. Sech. × Ihy	18	10	37.4 ± 8.7	8.0	15.3	76.7
Ta. Sech. × Ta. Ipo.	19	14	24.2 ± 4.6	5.6	88.3	6.1
Ta. Sech. × La. we	18	9	34.8 ± 5.8	31.9	57.4	10.7
La. × La.	49	13	59.3 ± 7.2	78.0	18.2	3.8
La.	55	28	42.8 ± 4.2	0.0	96.7	3.3
La. we × Ihy	20	12	51.6 ± 8.0	4.7	15.8	79.5
La. we × Ta. Ipo.	28	10	10.9 ± 2.5	6.1	74.4	19.5
La. we × Ta. Sech.	18	11	36.5 ± 9.9	80.3	16.8	2.9
T. urt. × Ihy	24	19	77.8 ± 8.7	0.0	95.8	4.2

Table IV gives information on hybrid fertility. For the F_2 and F_3 no accurate data on egg production could be obtained, because the egg-laying females were frequently disturbed, which affects egg production. However, a reasonable estimate of the sterile females and non-viable eggs was obtained. It is apparent that partial and even complete reproductive isolation exists between the strains studied, due to hybrid inviability, hybrid sterility and hybrid breakdown. It was not considered useful to erect new species as a result of this study, as no morphological differences were found between the strains. The fact that two completely incompatible strains were collected in the same area (Tananarive) from different plants suggests that more than one species is involved. The populations studied, therefore, should be grouped into a species complex.

The influence of the direction of the crosses on the F_1 (cf. reciprocal crossings between Ihosy × Tananarive Sechium and those between Ihosy × Louisiana we) suggests the existence of extra-chromosomal factors. These may affect embryonic development, resulting in a decreased percentage of viable eggs, or they may lead to disturbances in the gametogenesis, as was reported by Boudreaux (1963) for *T. urticae.*

INFLUENCE OF FERTILIZATION ON EGG PRODUCTION

A comparison was made of the egg production of *T. neocaledonicus* females mated by several males of their own strain or mated by several males of *T. urticae.* Under the same rearing conditions, the egg production of unmated females was determined, as well as that of females mated by males sterilized by means of treatment with X-rays.

Materials and method

Starting with the wild type Louisiana strain and a *T. urticae* strain found on *Sambucus nigra* L. in Voorne (Netherlands), the following rearings and crosses were made:

— females of *T. neocaledonicus* unmated
— females of *T. neocaledonicus* × normal males
— females of *T. neocaledonicus* × irradiated males
— females of *T. neocaledonicus* × males of *T. urticae*

Crossing experiments were carried out on detached leaves of *Phaseolus* sp. at a temperature of 25°, a humidity varying between 60—70% and under continuous light (van Zon & Helle, 1967).

For each cross, fifty female teleiochrysalids were placed on a single leaf, together with 25 males. After 24 hrs the females were placed separately on bean leaves. At the same time 50 unmated females were also placed individually on isolated leaves. Only the eggs laid during the first 14 days, and only from those females that had outlived this period, were counted and then destroyed. The percentage mortality and the sex ratio for each cross were determined in control experiments that were run at the same time. For irradiation a medical X-ray machine was used. Young males were treated at a dosage of 41 kr.

TABLE IV

Results of F_2 and F_3

Original cross	F_1-females		F_2			F_2-females		F_3		
♀ × ♂	No of ♀	Sterile ♀ (%)	Eggs per fertile ♀	non-viable eggs (%)	♀ progeny (%)	No of ♀	Sterile ♀ (%)	Eggs per fertile ♀	Non-viable eggs (%)	♀ progeny (%)
Ihy × Tul.	18	0	52.2	15.7	65	16	0	74.3	2.7	76
Ihy × Ta. Ipo.	4	100	—	—	—	—	—	—	—	—
Ihy × Ta. Sech.	18	0	39.4	29.2	86	20	25	50.8	21.7	70
Ihy × La. we	30	83	5.6	92.9	100	2	100	—	—	—
Tul. × Ihy	17	0	66.4	4.6	79	18	6	65.3	2.6	76
Ta. Ipo. × Ta. Sech.	5	100	—	—	—	—	—	—	—	—
Ta. Sech. × Ihy	18	17	18.2	84.6	90	12	33	44.6	82.0	90
Ta. Sech. × Ta. Ipo.	18	100	—	—	—	—	—	—	—	—
Ta. Sech. × La. we	30	100	—	—	—	—	—	—	—	—
La. we × Ihy	30	63	26.1	94.5	100	17	88	12.5	52.0	—
La. we × Ta. Ipo.	12	100	—	—	—	—	—	—	—	—
La. we × Ta. Sech.	18	17	16.1	79.0	74	18	22	35.7	80.3	74

Results and discussion

It appeared that *T. urticae* males exhibited a normal sexual activity towards the *T. neocaledonicus* females. Irradiation did not affect the mating behaviour of the males of *T. neocaledonicus*.

Table V demonstrates again that virgin females laid fewer eggs than fertilized ones. Crosses with irradiated males of *T. neocaledonicus* resulted in a much higher egg production, similar to the egg production of normally mated females, but a great number of non-viable eggs was found. It is assumed that insemination had

TABLE V

Comparison of partial egg production (first fourteen days of adult life) after rearing and crosses with several males

	Parents		F_1		
♀ × ♂	No of ♀	Mean egg production per ♀ ± t. S_m	♀ (%)	♂ (%)	Non-viable eggs (%)
La. unmated	34	46.3 ± 3.4	0.0	97.1	2.9
La. × La.	35	110.5 ± 7.2	78.9	18.7	2.4
La. × irrad. La.	38	94.6 ± 9.1	0.0	21.4	78.6
La. × *T. urticae*	12	113.8 ± 3.2	0.0	97.1	2.9

taken place but that fertilized eggs failed to hatch, because of the expression of dominant lethals. Notwithstanding the overlap of the confidence interval, an F-test showed a highly significant difference between the egg production of fertilized females of the original strain and that of fertilized females by irradiated males ($F = 7.56$, *cf.* 7.01 for $p < 0.01$). High percentages of non-viable eggs have already been found in crosses between Tananarive Sechium × Ihosy and Louisiana we. × Ihosy (Table III). In these two cases, however, a small female progeny was produced.

The crosses between *T. neocaledonicus* and *T. urticae* males resulted in a F_1 consisting of males only, the proportion of dead eggs was very low. Insemination apparently had taken place, as a considerable increase of egg production was observed, but no fertilization occurred. The increased egg production strongly suggests that this phenomenon does not depend on fertilization of the eggs, but is already induced by insemination.

At first, it was thought that sterilization of males by X-rays might help to explain the influence of fertilization on the increase of egg production. However, this appeared to be untrue. Still this test was extremely valuable for another objective, viz. biological control by an autocidal technique. The irradiated males appeared to be able to inseminate the females effectively, but no viable diploid offspring could be obtained.

GENERAL CONCLUSION

The five strains of *T. neocaledonicus* studied, collected in different biotopes from different plants, cannot be considered as a valid survey of the wide variety in strains of which this species consists. Nevertheless, certain conclusions can be drawn from our tests.

Using morphological criteria we failed to distinguish different strains, but physiological characteristics, such as longevity, egg production and consequently the net reproduction rate did indicate the existence of interstrain variation. This was strongly supported by the examination of the genetic affinities between the strains. The crosses between strains of *T. neocaledonicus* resulted either in incompatibility, or in hybrid inviability, hybrid sterility and hybrid breakdown.

As Helle (1965) pointed out for the *T. urticae* complex: the occurrence of a great interpopulational variability is the consequence of male haploidy. Favourable mutations have more chance to escape random forces, and can become fixed more easily in a population of an arrhenotokous species than in a population of a species of diploid sexes. There is an immediate interaction between mutation and selection in case of male haploidy, resulting in genetic drift-off of populations. Genetic variation, therefore, is expected to be inter-, rather than intrapopulational.

This special situation inclines one to consider *T. neocaledonicus* as a species complex in analogy to the *T. urticae* complex.

Differences in longevity and fecundity between mated and unmated females were found for all strains. The increase in egg production after mating seemed not to be dependent on fertilization, but seems to be related to the copulatory act itself. The increase in egg production might be induced by a stimulus caused by the filling of the bursa copulatrix. This reaction rapidly takes place if the male and the female originate from the same strain. A single mating then will be sufficient. However, our tests showed that when the partners were taken from different strains or when they belong to different species, several males were necessary to get this result.

Finally, attention is drawn to the importance of reproductive incompatibility between populations, for example between Tananarive Ipomoea and Tananarive Sechium. The few F_1-females resulting from these crosses were all completely sterile. In his proposition for biological control against spider mites by means of incompatible genes, Helle (1969) suggested that such a genetic control method would be feasible only if complete reproductive incompatibility is certain. Our preliminary experiments also gave hopeful perspectives for genetic control by means of releasing sterilized males. This last type of control, for instance against isolated tetranychid populations is, in our opinion, easy and certain, since the procedure may be used against any strain and in all probability will lead to a reduction of pest numbers.

RÉSUMÉ

UNE ÉTUDE COMPARATIVE DES SOUCHES DIFFÉRENTES DU COMPLEX TETRANYCHUS NEOCALEDONICUS *ET EXPÉRIENCES SUR LA STÉRILISATION DES MÂLES AUX RAYONS X*

A la suite d'une étude morphologique concluant à l'homogénéité d'un grand nombre de prélèvements de *Tetranychus neocaledonicus*, dans plusieurs régions de la zone intertropicale, les auteurs proposent 3 séries d'expériences portant sur la physiologie et la compatibilité de quelques souches de ce taxon.

La première série d'essais, porte sur la comparaison de la longévité et de la fécondité de femelles fécondées ou non, appartenant à 4 souches prélevées à Madagascar et à une souche originaire de Louisiane (U.S.A.). Pour ces souches élevées dans des conditions identiques, les femelles fécondées pondent davantage et vivent moins longtemps que les femelles vierges. Les graphiques fournis et les tests statistiques employés, montrent que suivant leur appartenance à l'une des 5 souches, les femelles étudiées ont une longévité propre et présentent du point de vue de la reproduction, des différences très nettes.

La seconde série d'expériences a pour but de vérifier la compatibilité entre les différentes souches malgaches précédentes, aussi bien entre elles qu'avec la souche Louisiane "white eye", marquée génétiquement et dérivant de la souche Louisiane précédente. Un croisement a été également tenté entre une souche de *T. neocaledonicus* et une souche de *T. urticae*. Huit croisements ont été ainsi réalisés avec chaque fois le croisement réciproque. La composition des F_1, F_2 et F_3 est analysée. Une très forte agressivité des mâles à l'égard des jeunes femelles est chaque fois constatée. Le croisement de *T. neocaledonicus* avec *T. urticae* n'entraîne aucune hybridation. Entre les souches de *T. neocaledonicus* il y a formation de femelles, dans l'un des 2 sens au moins mais les femelles produites en F_1, se révèlent stériles dans 5 cas sur 12 et peu de souches semblent vraiment compatibles au-delà de la F_3. Finalement, les différences entre les souches du point de vue physiologique et les incompatibilités génétiques, amènent les auteurs à considérer qu'il y a, en fait, plusieurs espèces de *T. neocaledonicus* devant être groupées en un complexe. Ces croisements permettent aussi de remarquer, que l'abaissement de la longévité des femelles fécondées, dépend étroitement du taux de fécondation des œufs.

La troisième série d'expériences tente d'expliquer l'augmentation de la production d'œufs chez les femelles fécondées. Les pontes des 14 premiers jours de vie, de femelles de la souche Lousiane fécondées ou non, sont comparées à celles de femelles fécondées, par des mâles de la même souche, irradiés aux rayons X ou par des mâles de *T. urticae*. La technique d'élevage employée ici permet l'intervention de plusieurs mâles. L'analyse de la F_1 et la quantité d'œufs produits à la suite du croisement avec les mâles de *T. urticae*, prouvent que l'augmentation de la ponte est dûe à un phénomène indépendant du taux de fécondation des œufs.

Les auteurs signalent, en conclusion, que les incompatibilités entre les différentes souches de *T. neocaledonicus* pourraient être utilisées en lutte biologique par voie génétique, mais que la stérilisation des mâles aux rayons X, obtenue au cours de la dernière série d'expériences, aboutit aux mêmes résultats et paraît plus sûre.

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