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Mechanical and Aphid Transmission of an Ivory Coast Strain of Groundnut Rosette Virus

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Groundnut rosette was first observed in 1907 by ZIMMERMANN. STOREY and BOTTOMLEY (1928) described the transmission of its causal agent(s) by the aphid, *Aphis craccivora* Koch., and other authors transmitted several strains of groundnut rosette virus using several races of *Aphis craccivora* (STOREY and RYLAND 1955, BRUNT and BONNEY 1964, WATSON and OKUSANYA 1967). They did not, however, distinguish exactly between the main stages of transmission: acquisition, inoculation and retention, nor did they test for transmission to progeny.

The combined works of STOREY and RYLAND (1950), BRUNT and BONNEY (1964), OKUSANYA and WATSON (1966), and HULL and ADAMS (1968) showed the existance in diseased plants of two entities, groundnut rosette virus (GRV), which was transmitted by inoculation with sap, and its assistor virus (GRAV), which was not mechanically transmissible. GRAV was not characterized, but was separated from GRV (HULL and ADAMS 1968).

The present paper describes the first stages of the study on an Ivory Coast strain of groundnut rosette virus and attempts to separate GRV and GRAV.

Materials and Methods

The IRHO[1]) cultivar Te3 was used in all the experiments. It was more susceptible to the groundnut rosette disease than other local, American or I.R.H.O. varieties.

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All test plants were grown in screenhouses, where temperatures ranged from 26 to 35 °C during the day. Relative humidity was always 95—100 % and daylength ca. 12 h. Keeping them in darkness before inoculation did not increase their susceptibility. *Chenopodium, Trifolium, Melilotus* and *Medicago* spp. received 6 h extra light, provided by fluorescent tubes.

Plants affected by groundnut rosette disease were observed in fields near The O.R.S. T.O.M. Centre, in the south of the Ivory Coast. The diseased plant chosen as a source of virus showed the symptoms typical of groundnut chlorotic rosette disease described by STOREY and RYLAND (1957), and different from those of green rosette, vein banding, mosaic or ringspot diseases (KLESSER 1968a and 1968b). Aphids fed on this plant were used to inoculate 30 Te3 seedlings. The most typical diseased plant was used as a source of inoculum for insect transmission to another group of 30 groundnut seedlings. The whole process was repeated five successive times, and, in the group inoculated last the most typical diseased plant was chosen as the inoculum for transmission experiments and called the Ivory Coast strain.

HULL and ADAMS' (1968) method was modified and used in all our experiments. Inocula were prepared by grinding infected leaves of *Arachis hypogaea* using mortars at —16 °C, in 0.05 M potassium phosphate buffer at pH 7.3, containing 0.01 M sodium diethyldithiocarbamate and 10 mg/ml magnesium bentonite. Inocula were rubbed on Carborundum-dusted leaves of test seedlings. One month later, back inoculations were made to Te3 groundnut plants to detect symptomless infections. All plants were used in host or transmission studies when young and growing vigorously. Groundnut plants were inoculated 8—12 days after sowing when they had 2—3 leaves. Only *Chenopodium* spp. were used when they were older, just before flowering.

Aphid transmission experiments were done in the laboratory with an Ivory Coast culture of *Aphis craccivora* Koch. maintained on *Chenopodium quinoa* Willd. or *Vigna unguiculata* (L.) Walp. cv. *sinensis*, two species which were not systemically susceptible to the rosette disease agents. The aphids were cultured in an air-conditioned room to avoid the attacks of *Entomophtora fresenii* (ROCKWOOD 1950).

For the host range study, 10 late instar apterous aphids, reared on diseased groundnut, were transferred to each test plant: after 2 days they were killed with an insecticide, Systoate. The plants were then transferred in screenhouses and sprayed with an insecticide once a week. After 1 month, back tests were made to groundnut to detect symptomless infections, using aphids and by inoculation with leaf extracts.

In vector relations studies, insects were killed manually, because of the too slow action of insecticides.

In attempts to separate different components occurring in rosette-diseased plants, the possibility was tested that they differ in host ranges or transmissibility by mechanical inoculation, or have different vector relations. The plants that remained symptomless in experiments, in which the aphid access periods were varied, were each colonised by 10 virus-free late instar apterous aphids. After a 6 h acquisition access period, which was considered long enough for some aphids to acquire the postulated assistor virus, the insects were transferred to mechanically inoculated groundnut plants showing rosette symptoms. After 48 h the aphids were transferred to healthy plants.

In all the experiments different control tests were made. For the mechanical transmissions and the host range studies, groundnut seedlings were also inoculated in order to control infectivity of diseased-groundnut extracts, and five of ten plants of each species were inoculated with an extract, obtained by the method described, from healthy groundnut plants to confirm lack of contamination.

In the experiments on vector relations, the insect culture was always checked for freedom from virus, and, in the host range studies the infectivity of the aphids was checked by caging specimens on 10 healthy plants.

In the host range studies and in the experiments on vector relations, back inoculations were made on groundnut seedlings mechanically and by insect.

In the experiments on transmission to progeny, control tests included the transfer to healthy groundnut plants of first instar apterous aphids which had fed on infected plants.

Results

Plant hosts and symptomatology

The species infected by mechanical or aphid inoculations are listed in Table 1.

Table 1
Host range tests with groundnut rosette virus

Family/species	Mechanical inoculation			Aphid inoculation		
	result	MI	II	result	MI	II
Chenopodiaceae						
Chenopodium amaranticolor	LLN	0	0	0	0	0
C. murale	LLC	0	0	0	0	0
C. quinoa	LLCN	+	0	0	0	0
Leguminosae						
Arachis hypogaea	CR	+	0	CR	+	+
Centrosema plumieri	m	0	0	0	0	0
Crotalaria juncea	d	0	0	0	0	0
Phaseolus mungo	LLN	0	0	0	0	0
Tephrosia vogelii	0	0	0	M	0	0
Trifolium repens	m	+	0	m	+	0
Stylosanthes gracilis	M	+	0	M	+	0
S. mucronata	M	+	0	M	+	+
Solanaceae						
Physalis floridana	m	0	0	m	0	0

0: plants tested but no infection; +: plants tested and disease recovered to groundnut plants; MI: back test to groundnut plants by mechanical transmission; II: back test to groundnut plants by insect transmission; LLC: chlorotic local lesions; LLN: necrotic local lesions; LLCN: chlorotic then necrotic local lesions; CR: systemic chlorotic rosette; M: systemic mottle; m: systemic mottling; d: systemic dotting.

Moreover many species were tested by mechanical or aphid transmission and were not infected (The number of plants tested of each species is given in parentheses):

Aizoaceae: *Tetragonia expansa* (36): Amaranthaceae: *Amaranthus caudatus* (8), *Celosia cristata* (12), *Gomphrena globosa* (12). Apocynaceae: *Vinca rosea* (12). Chenopodiaceae: *Beta vulgaris* (12), *Chenopodium album* (20). Compositae: *Callistephus sinensis* (16), *Calliopsis tinctoria* (6), *Zinnia elegans* (6). Cucurbitaceae: *Cucumis sativus* (12), *Cucurbita pepo* (12). Cruciferae: *Brassica oleracea* (6). Leguminosae: *Alysicarpus longifolius* (6), *Canavalia ensiformis* (12), *Cassia tora* (12), *C. occidentalis* (12), *Centrosema pubescens* (60), *Crotalaria pallida* (12), *C. usuramoensis* (12), *Glycine max* (36), *Indigofera hirsuta* (24), *Lupinus vulgaris* (12), *Medicago sativa* (24), *Melilotus alba* (12), *Phaseolus lunatus* (24), *P. lathyroïdes* (24), *P. vulgaris* (24), *Pisum sativum* (24), *Sesbania sesban* (12), *Trifolium pratense* (6), *Vicia faba* (48), *Vigna*

sesquipedalis (24), *V. unguiculata* (24), *V. unguiculata* cv. *sinensis* (24). Malvaceae: *Hibiscus esculentus* (24), *Gossypium hirsutum* (24).

Scrophulariaceae: *Anthirrinum majus* (24). Solanaceae: *Capsicum annuum* (12), *C. frutescens* (12), *Datura metel* (12), *D. inoxia* (12), *Lycopersicon esculentum* (24), *Nicotiana tabacum* cv. Samsun (12), *N. tabacum* cv. Xanthi (12), *N. tabacum* cv. White Burley (12), *N. glauca* (12), *N. glutinosa* (24), *N. clevelandii* (24), *N. rustica* (48), *Petunia rosea* (24), *P. nana-compacta* (24), *P. hybrida* (24), *Physalis alkekingie* (24).

Vector relations

a. Acquisition access period

Late instar apterous aphids were starved for 2—3 h, then allowed various acquisition access periods. They were then transferred to healthy groundnut seedlings where they fed until they died. Transmission occurred following acquisition access period of 4.5 h or longer, the percentage of transmission increasing from 13 to 93 as the access time increased from 4.5 to 24 h (Table 2).

Table 2
Effect of acquisition access time on transmission of groundnut rosette virus

Acquisition access time (h)*)	1	2	3	4.5	6	12	18	24	48
No. of plants infected (out of 30)	0	0	0	4	8	12	20	28	30
% plants infected	0	0	0	13	27	40	67	93	100

*) Acquisition access time before an inoculation time of several days (up to insect death). Ten late instar apterous aphids per plant.

b. Inoculation access period

Using late instar apterous aphids reared on diseased groundnut plants, transmission occurred with inoculation access periods of 3 min or longer, 10 min giving 92 % transmission (Table 3).

Table 3
Effect of inoculation access time on transmission of groundnut rosette virus

Inoculation access time (min)*)	0.5	1	3	5	10	15	20
No. of plants infected (out of 12)	0	0	2	8	11	12	11
% plants infected	0	0	17	67	92	100	92

*) Inoculation access time after an acquisition access time of several days: late instar apterous aphids borne and reared on diseased plants. Ten apterous aphids per plant.

c. Latent period

After an acquisition access time of 4.5 h, apterous aphids could not immediately transmit groundnut rosette virus. A latent period was necessary

before the virus could be transmitted. To determine this, late instar apterous aphids were starved for 2—3 h, then allowed an acquisition access period of 4.5, or 24 h. They were then transferred to non-susceptible plants (*Vigna unguiculata*) for specified times, and finally transferred to healthy groundnut seedlings for 10 min, after which they were killed manually. After a 4.5 h acquisition access period, a latent period of 18 h was necessary before the virus could be transmitted. After a 24 h acquisition access period, this latent period decreased to 2 h (Table 4).

Table 4
Latent period in transmission of groundnut rosette virus*)

Interval between acquisition and inoculation (h)	1	2	3	4.5	6	12	18	24	48
Transmission after acquisition access of 4.5 h	0/15	0/15	0/15	0/30	0/30	0/45	2/60	6/60	10/60
Transmission after acquisition access of 24 h	0/15	1/15	2/30	5/30	8/30	12/30	20/30	28/30	27/30

*) Inoculation access time of 10 min and 10 late instar apterous aphids per plant.

d. Retention period

This period was studied on last instar apterous aphids reared on diseased groundnut plants. Groups of aphids were transferred each day to new healthy plants, after being brought together and devided in new groups. Table 5 shows that the virus was retained by the aphids until they died, and it was transmitted on the fifteenth day.

Table 5
Retention of groundnut rosette virus by *Aphis craccivora*

No. of daily transfers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No. of aphids per plant	12	11	10	8	8	6	6	6	5	5	5	4	3	2	1
No. of infected plants	9	12	9	8	5	4	4	3	3	4	2	3	2	1	2
	30	29	19	13	7	6	6	6	6	6	6	6	3	2	2

e. Transmission to progeny aphids

To test for transmission of virus from mother aphids to their viviparous progeny, first instar apterous aphids, as they were borne, were collected from infective mothers and, before they could feed, 10 such aphids were placed on each healthy groundnut plant and kept there until they died. Thirty seedlings were tested. One month after exposure to these aphids, none out of 30 seedlings had developed rosette symptoms. In contrast with first instar apterous insects that had fed on infected plants, six out of 30 seedlings became diseased.

Experiments to separate the two components

The first experiment was the host range study. In these tests groundnut rosette virus was separated from its assistor by both mechanical and insect inoculations and could replicate alone; but the assistor virus was never obtained on its own. Groundnut rosette virus was the only virus in all the mechanically-inoculated plants and in two insect-inoculated species: *Trifolium repens* and *Stylosanthes gracilis*, and perhaps in two other species from which the disease could not be recovered: *Tephrosia vogelii* and *Physalis floridana* because back tests from these plants to groundnut seedlings by insect inoculation failed always.

The second experiment consisted of back inoculation tests from the symptomless plants in the studies of acquisition, inoculation, latent and retention periods. Aphids were confined first on these plants, then on mechanically inoculated groundnut plants with rosette symptoms, and finally transferred to healthy groundnut plants. 657 groundnut symptomless seedlings were tested and rosette symptoms never developed. In the transmission period studies, the plants were either diseased and contained both virus components (back transmission was possible by mechanical and insect inoculation), or the plants were symptomless and did not contain groundnut rosette virus (mechanically back inoculation alway failed); these symptomless plants contained perhaps groundnut rosette assistor virus, but this was not recovered.

This last procedure was also used to try to recover the assistor component from some symptomless species (number of tested plants in parentheses): *Chenopodium album* (20), *C. quinoa* (20), *C. amaranticolor* (20), *Tetragonia expansa* (20), *Centrosema plumieri* (20), *C. pubescens* (20), *Glycine max* (20), *Indigofera hirsuta* (12), *Phaseolus mungo* (20), *Sesbania sesban* (12), *Trifolium pratense* (6), *Capsicum frutescens* (12), *Datura metel* (12), *Nicotiana tabacum* cv. Xanthi (12), *N. glauca* (6), *N. clevelandii* (12), *Petunia rosea* (20). The assistor component was never recovered.

Discussion

Mechanical and insect transmission gave a host range slightly different from the lists published by others for many isolates of groundnut rosette virus. However tests did not include *Sesbania aegyptica, Trifolium incarnatum* and *Stylosanthes juncea*. Moreover the Ivory Coast virus strain does not seem to infect *Glycine max* and *Petunia nana-compacta*. *Chenopodium quinoa* is a good local lesion host, from which groundnut rosette was recovered to groundnut seedlings. *Stylosanthes* is an interesting genus; *S. mucronata* accepts the two components by insect transmission, but *S. gracilis* is only infected by the rosette virus though not by the assistor component (HULL and ADAMS 1968).

Aphid transmission studies in general confirm the results of WATSON and OKUSANYA (1967) and other authors, and they give additional data on latent, inoculation and retention periods. A long acquisition access period was needed

to give efficient transmission; however, an acquisition access time of 4.5 h was enough to transmit the rosette disease agent and the optimal acquisition access period was approximately 24 h. The latent periods ranged from 2 to 18 h, depending on the acquisition access period: a short acquisition access period of 4.5 h entailed a long latent period of 18 h, whereas an acquisition access period of 24 h entailed a latent period of only 2 h. In fact, an acquisition access time of 24 h includes most of the latent period and the more realistic latent period is that (18 h) calculated after the shortest acquisition access time. The inoculation access time has no influence on this estimate because only 10 min was needed for efficient inoculation. These vector relations of the rosette virus are typical of persistent viruses, as is the retention of the virus by aphids until they died. Indeed the virus may replicate in the insect. In preliminary tests the virus also persisted in second and third instar apterous aphids until they died. No transmission to progeny aphids was observed, although rare transmission cannot be excluded.

The results confirm that the virus (groundnut rosette virus) that can be transmitted by inoculation with sap thereby loses its aphid transmissibility (OKUSANYA and WATSON 1967, HULL and ADAMS 1968). A possible interpretation of this finding is that naturally-infected plants with rosette disease contain two entities, groundnut rosette virus (GRV) and an assistor virus (GRAV), which is not sap-transmissible (OKUSANYA and WATSON 1967, HULL and ADAMS 1968). However, unlike HULL and ADAMS (1968), either I failed to obtain a pure culture of the postulate assistor virus, or I failed to recover the postulate assistor virus if a pure culture was obtained. Only the method used to recover GRAV in these experiments differs from this of HULL and ADAMS (1968). Instead of grafting simultaneously GRV-infected plants and GRAV-infected plants on groundnut seedlings and then showing that rosette disease was insect transmissible, we tried to recover GRAV directly by insect transmission. It seems that it was not possible to recover GRAV by this way. Probably GRV was essential to protect GRAV in order to be acquire by insect. Further work is needed to clarify the nature of the assistor virus and its relation with groundnut rosette virus.

Summary

The minimum periods for acquisition and inoculation of an Ivory Coast chlorotic strain of groundnut rosette virus by *Aphis craccivora* were 4.5 h and 3 min respectively. There was an important latent period in the aphid and the minimum time for transmission was 22.5 h. The virus persisted in the aphids until they died but was not passed to progeny.

A virus transmitted by inoculation of sap from rosette-affected plants induced rosette symptoms but was no longer transmissible by *A. craccivora*. The existence of two components was confirmed, but the assistor virus was not recovered by insect transmission.

Résumé

Transmission par voie mécanique et par puceron d'une souche ivoirienne du virus de la Rosette de l'Araclude

La transmission par *Aphis craccivora* d'une souche chlorotique du virus de la rosette de l'Arachide a été étudiée en Côte d'Ivoire. La période minimale d'acquisition du virus est de 4 h 30 min et la période minimale d'inoculation du virus de 3 min. La periode de latence est importante: 22 h 30 au minimum. Le virus persiste dans l'insecte jusqu'à la mort de celui-ci. Il ne semble pas exister de transmission transovarienne.

Un virus a été transmis par voie mécanique à partir d'extraits de plantes naturellement infectées par la rosette; par ce mode de transmission le virus a perdu la capacité d'être transmis par *A. craccivora*. L'existence de deux composants dans le virus est confirmée; cependant, le virus auxilliaire n'a pu être récupéré par transmission par insecte.

Zusammenfassung

Mechanische und Aphidenübertragung eines Stammes des Erdnuß-Rosetten-Virus der Elfenbeinküste

Bei der Übertragung des Erdnuß-Rosetten-Virus durch *Aphis craccivora* ist eine Aufnahmezeit von 4,5 Stunden und eine nachfolgende Latenzperiode von wenigstens 22,5 Stunden erforderlich. Die Inokulationszeit beträgt mindestens 3 Minuten. Das Virus bleibt im Insekt persistent bis zu dessen Tode, wird aber nicht auf die Nachkommenschaft übertragen. Wurde das Virus mit dem Saft natürlich infizierter Pflanzen mechanisch übertragen, ließ es sich nicht durch *A. craccivora* weiter übertragen. Es wird daher die Existenz von zwei Komponenten in Betracht gezogen, jedoch ließ sich das vermutete Begleitvirus nicht nachweisen.

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