Aphid Transmission of the Viruses Causing Chlorotic Rosette and Green Rosette Diseases of Peanut in Nigeria

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ABSTRACT

Misari, S. M., Abraham, J. M., Demski, J. W., Ansa, O. A., Kuhn, C. W., Casper, R., and Breyel, E. 1988. Aphid transmission of the viruses causing chlorotic rosette and green rosette diseases of peanut in Nigeria. Plant Disease 72:250-253.

Transmission of the causal agents, isolated in Nigeria, of two forms (chlorotic rosette and green rosette) of groundnut rosette by the cowpea aphid, *Aphis craccivora*, was compared in concomitant experiments. Aphids could acquire groundnut rosette virus (chlorotic) (GRV-C) within 4 hr and groundnut rosette virus (green) (GRV-G) within 8 hr. For both GRV-C and GRV-G, latent periods varied from 1 to 11 days; median latent periods were 26.4 and 38.4 hr for GRV-C and GRV-G, respectively. After a 24-hr latent period, viruses of both diseases could be transmitted within 10 min (inoculation access period). Multiple plant infections (serial transfers) tended to occur more frequently with GRV-C than with GRV-G. Maximum retention time was the lifetime of aphids (about 14 days) for both viruses. Overall transmission efficiency of GRV-C and GRV-G increased from 26 to 31 to 49% when one, two, and five aphids, respectively, were allowed to feed on test plants. No major differences in transmission efficiency of GRV-C and GRV-G were detected, however, with increased numbers of aphids.

Groundnut rosette is a destructive disease of peanut (*Arachis hypogaea* L.) that is widespread in Africa (3,14,21). The disease is common in northern Nigeria, where it is considered one of the main reasons for the decline in peanut production in the country. A rosette epidemic in Nigeria in 1975 destroyed an estimated 0.7 million hectares of peanut (21). An epidemic occurred again in 1985, with near total loss of yield in some regions of the country.

Chlorotic rosette and green rosette are two distinct but similar diseases of peanut. Green rosette was the only disease in the 1975 epidemic, and it continues to be the most important virus disease of peanut in Nigeria. The incidence of chlorotic rosette, however, has been increasing during the last 5 yr and may be as high as 10–20% in some fields (surveys by authors, *personal observation*). Storey and Ryland (16) speculated that green rosette and chlorotic rosette are strains of the same virus. The relationship between the two diseases has not been determined, but the viral causal agents in question do seem to possess similar properties and characteristics (1,9,10).

Groundnut rosette virus (GRV) appears to be responsible for the rosette disease symptoms (5,9). Therefore, variants of GRV probably cause chlorotic rosette and green rosette. GRV depends on groundnut rosette assistor virus (GRAV) for aphid transmission (5,8), and GRAV is believed to cause no symptoms in peanut (5). GRAV has not been isolated and characterized, but it reacts with the antisera of several luteoviruses (1,9). The same isolate of GRAV may be associated with both green rosette and chlorotic rosette. Although the exact nature of GRV is unknown, the surmise is that it lacks a coat protein and its nucleic acid is encapsidated by the coat protein of GRAV for aphid transmission (1,10). Presumably, the aphids can transmit GRV (C or G) singly or concomitantly with GRAV. In the field, we have found symptomless plants with GRAV but, to date, no diseased plants with GRV alone (Ansa, *unpublished*). For our studies, aphid transmission of GRV was judged to have occurred when the distinct and unique symptoms of either GRV-C or GRV-G developed on peanut plants.

The black cowpea aphid, *Aphis craccivora* Koch, is the best known, if not the only significant, vector of the groundnut rosette viruses transmitted in a persistent manner (15,20). Some virus-vector relationships of green rosette isolates from Nigeria and chlorotic rosette isolates from East Africa were studied in England by Okusanya (7). She showed that a Nigerian population of *A. craccivora* was capable of transmitting the East Africa isolate of GRV (chlorotic) (GRV-C) and the Nigerian isolate of GRV (green) (GRV-G). The populations of aphids from East Africa, however, failed to transmit GRV-G from Nigeria.

The increasing incidence of chlorotic rosette in recent years has caused considerable concern in Nigeria. A few peanut cultivars believed to be resistant to green rosette became severely diseased with chlorotic rosette in 1983 and 1985. Furthermore, simultaneous and challenge inoculation studies indicate that GRV-C is more aggressive (initiates infection faster and overcomes a previous early infection with GRV-G) than GRV-G (authors, *unpublished*). We therefore deemed it desirable to make direct comparisons of the virus-vector relationships associated with the chlorotic rosette and green rosette diseases in Nigeria.

MATERIALS AND METHODS

Rosette viruses. GRV-C and GRV-G and their assistor virus, GRAV, were obtained from field-infected plants in peanut experimental plots at the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The viruses were maintained in susceptible peanut genotypes MK 374 and Samaru 38 by periodically transmitting them to young seedlings via aphids (*A. craccivora*). Test plants were raised from seeds sown directly into 6.5- to 12.5-cm-diameter pots containing soil. Test seedlings usually were inoculated within 5 days of germination.

Unless otherwise stated, 10–25 test plants per treatment were used in each experiment, which was repeated at least three times. Uninoculated control plants were used in each test.

Aphid manipulation. Aphid stock cultures were initiated from adult apterae from cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) plants in the field. Apterae were placed in a petri dish lined with filter paper moistened with water. The nymphs, deposited overnight, were placed on healthy peanut seedlings and allowed to develop to adults. Aphid stocks were established by serially

Research was supported by funds from the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria; by Peanut CRSP, U.S. AID Grant DAN-4048-G-SS-2065-00; and by the Commission of the European Communities, Grant TSD-A-66-D.

Accepted for publication 26 October 1987 (submitted for electronic processing).

transferring the larvipositing apterae at 24-hr intervals to peanut seedlings. Since none of the plants used for rearing the aphids showed any rosette or other viruslike symptoms, the aphids were assumed to be free from either GRV-C or GRV-G.

Aphids were transferred to individually caged test plants. After the prescribed access periods, the aphids were removed and the plants were sprayed with an insecticide. The plants were then transferred to the greenhouse and observed for symptom development for 3–4 wk. All tests were generally conducted concurrently with GRV-C and GRV-G.

Acquisition access period. Batches of about 100 first- to second-instar nonviruliferous aphids (average age = 36 hr) were allowed to feed separately on 11 similar peanut plants with chlorotic rosette or green rosette for periods varying from 0.25 to 60 hr. Immediately after each acquisition access period, single aphids from each group were transferred to individual healthy peanut test plants and allowed an inoculation access period of another 24 hr. Serial transfers were made to new test plants every 24 hr until the aphids died.

Inoculation access period. Nonviruliferous aphids, 3–4 days old, from the stock culture were allowed an acquisition access period of 48–72 hr on infected source plants. The aphids were pooled and then confined singly or in groups of five on 10–25 individual test plants. After inoculation access periods varying from 5 to 40 min and from 6 to 48 hr, aphids were removed.

Latent period and retention. In some cases, the latent period was studied as an extension of the acquisition access period tests. Second-instar nymphs were allowed an acquisition access period of 24 hr. The aphids were individually caged on separate test seedlings and serially transferred at 24-hr intervals to fresh sets of plants. From each set of transfers, the cumulative percentage of first transmissions was recorded for determination of the median latent period by the method described by Sylvester and Osler (17). Retention of persistence was noted as the time period from leaving source plants to the last successful transmission in the serial transfers.

Vector number and transmission. Single aphids and groups of two and five aphids reared on GRV-C and GRV-G source plants were caged on two susceptible peanut genotypes to compare their efficiency of transmission. After a 48-hr inoculation access period, the aphids were removed. Ten test plants were used for each peanut genotype, for each disease, and for each variation in the number of aphids per plants. The experiment was repeated three times.

In addition to comparing the transmission of GRV-C and GRV-G, the hypothesis that the effect of a given aphid feeding on a test plant during the inoculation access period is independent of any other aphid feeding on the same plant was tested by a probability of infection formula previously used by Watson (19) and Storey (13). The probability of infection (P) as a result of feeding by a given aphid is assumed constant and independent of whether or not other aphids are feeding on the plant. The probability q of any aphid not transmitting virus is $q = (1-p)$. The probability of n aphids of all aphids feeding at once without transmitting would be q^n.

RESULTS

Acquisition access period. In several experiments, GRV-C was acquired faster than GRV-G (Table 1, Fig. 1). GRV-C was acquired as quickly as 30 min in one experiment but usually required 4 hr. GRV-G required 8 hr in all experiments. The percentage of successful transmissions increased with longer acquisition periods for both GRV-C and GRV-G (Table 1). From 24 to 60 hr, single aphids could transmit the viruses to 40% or more of the test plants. Although the short acquisition periods were more favorable for GRV-C than for GRV-G, periods from 8 to 60 hr were similar for both viruses.

Inoculation access period. Both GRV-C and GRV-G could be transmitted within a 10-min inoculation access period but not within 5 min (Table 2). The number of successful transmissions increased with longer inoculation periods, up to 48 hr. Transmission was similar for GRV-C and GRV-G regardless of the length of the inoculation access period or the number of aphids per plant.

Latent period. The term "latent period" is imprecise because acquisition could occur any time during the 24-hr acquisition access period. For these comparative studies between GRV-C and GRV-G, therefore, we selected the midpoint of the acquisition access period as the beginning of the latent period. Both GRV-C and GRV-G required latent periods of 1–6 days in the vector before transmission could be achieved (Fig. 1). In one instance, however, transmission of GRV-G by one aphid did not occur until 11 days after an acquisition access period. About 60% of the first transmissions of both GRV-C and GRV-G had occurred by the second day. In general, the latent period was shorter with longer acquisition access periods: 4 days or more with 8 hr or less of an

Table 1. Transmission of the viruses causing chlorotic rosette and green rosette diseases to peanut seedlings (Samaru 38) by single aphids (*Aphis craccivora*) with varying acquisition access periods[a]

Acquisition access period (hr)	Percentage of plants infected	
	Chlorotic rosette	Green rosette
0.25	0	0
0.5	2	0
1	0	0
2	0	0
4	9	0
8	22	18
12	29[b]	9[b]
24	47	40
36	47	48
48	47	58
60	67	60
Overall average	25	21

[a] Data combined from three experiments—two with 10 plants per disease per time period and one with 25 (45 total).
[b] Only 35 plants inoculated.

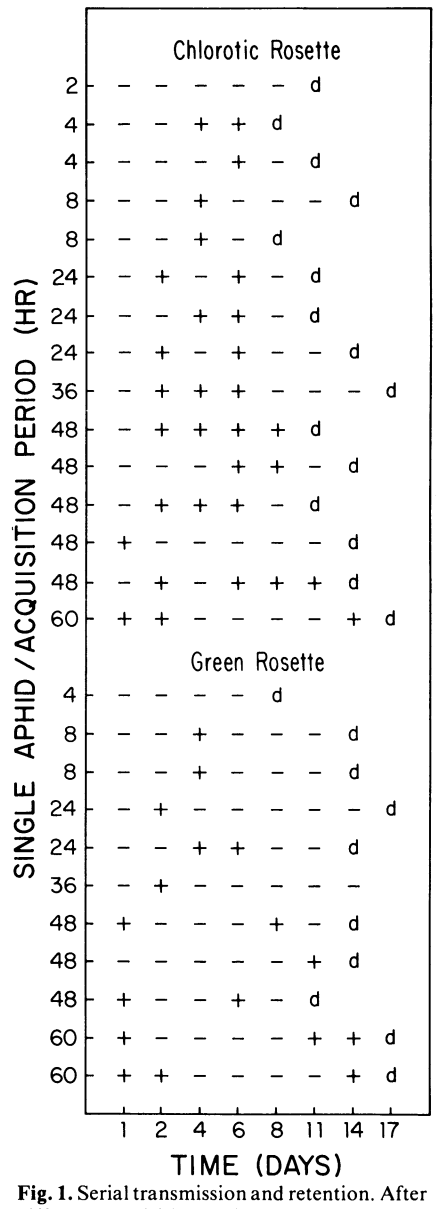

Fig. 1. Serial transmission and retention. After different acquisition periods on peanut plants showing symptoms of chlorotic rosette or green rosette, single aphids (*Aphis craccivora*) were transferred to individual healthy peanut plants. Thereafter, the same single aphids were serially transferred to fresh healthy plants at differing time periods of 1–17 days. + = Infection, − = no symptoms, d = aphids dead.

acquisition period, 2 days with 24 hr, and 1 day with 48 hr or more.

When results were combined from several acquisition access period tests, the median latent periods were determined to be 26.4 and 38.4 hr for GRV-C and GRV-G, respectively. Thus, 50% of the aphids that eventually became infective could transmit the viruses within 1–1.5 days.

Serial transmission and retention. Serial transmission studies demonstrated that single aphids could transmit GRV-C and GRV-G to more than one peanut plant. Multiple plant infections occurred with 71 and 50% of single aphids carrying GRV-C and GRV-G, respectively (Fig. 1).

Transmission during daily serial transfers was not continuous but erratic for both GRV-C and GRV-G. After a successful first transmission, skips varied from 1 to 4 days before a second transmission occurred (Fig. 1). In the Figure 1 study and two other similar studies, more than 50% of the first transmissions occurred during the first 2 days after the end of the acquisition access period.

The maximum retention period for both GRV-C and GRV-G was 14 days, and the average retention times for GRV-C and GRV-G were 6.6 and 6.9 days, respectively (Fig. 1). The data tend to demonstrate that increasing the acquisition access period from 4–8 hr to 24–60 hr shortens the latent period and increases the number of plants that will become infected by single aphids.

Vector number and transmission. Transmission of both GRV-C and GRV-G to susceptible peanut genotypes was similar regardless of the number of aphids feeding on test plants. The overall transmission efficiency was about 35% for each isolate, and efficiency increased from 26 to 31 to 49% when one, two, and five aphids, respectively, were allowed to feed on single test plants (Table 3). Observed transmission data and expected probability of transmission indicate that fewer plants than expected became infected when multiple aphids per plant were compared to single aphids (Table 3) (13,19).

DISCUSSION

Our concomitant comparison studies showed a tendency for GRV-C to be more readily transmitted by *A. craccivora* than GRV-G. For GRV-C, the acquisition access period and the latent period were shorter and multiple plant infections by single aphids occurred more frequently. Differences between GRV-C and GRV-G occurred during short acquisition access and inoculation access periods; when each period was 24 or 48 hr or longer, however, differences usually were not observed. We conclude that the basic transmission mechanisms of the virus-vector relationships are the same or very similar for GRV-C and GRV-G. This conclusion is consistent with the possibility that the nucleic acid of GRV (any isolate) is encapsidated in the coat protein of GRAV (1,9,10). Virus transmission by vectors depends on the interaction of the vector and the viral coat protein in those virus-vector combinations that have been studied (4,11).

One possible explanation for the observed transmission differences between GRV-C and GRV-G is that concentration of GRV-C may be greater than that of GRV-G in the host peanut (suggested by simultaneous and challenge inoculation studies by the authors, *unpublished*). Rosette-diseased plants would be expected to have a mixture of GRAV and GRV particles, and the ratio of the two viruses in the mixture could differ on the basis of the amount of viral RNA produced by either GRV-C or GRV-G. A shorter acquisition access period would allow an "infective unit" to be acquired more readily when in the highest concentration. Longer acquisition access periods would tend to nullify the advantage of GRV-C, and that was the result observed.

At this time, it is premature to speculate on any epidemiological implications regarding the transmission differences between GRV-C and GRV-G. The incidence of chlorotic rosette is indeed increasing in northern Nigeria. This could be explained by a gradual buildup in the source of primary inoculum, which has not been identified for either GRV-C or GRV-G.

Increasing the number of aphids (that had fed previously on infected plants) per susceptible peanut plant increased the number of successful transmissions. The number of transmissions was lower than expected, indicating clearly that the infections were not the result of an accumulation of subinfective doses from more than one aphid (13,19). We have no explanation for the fewer than expected transmissions, although Storey (13) suggested that inhibitory effects between aphids and plant variability could be responsible. For the objectives of this study, no differences in transmission efficiency were observed between GRV-C and GRV-G.

Several studies were conducted with *A. craccivora* and the groundnut rosette viruses between 1955 and 1980 (2,6,12, 15,18). In all cases, the virus-vector relationship was regarded as persistent. However, time periods for specific tests varied. For example, the acquisition

Table 2. Effect of varying inoculation access feeding periods on transmission of the viruses causing chlorotic rosette and green rosette diseases by *Aphis craccivora* to peanut seedlings (Samaru 38) after a 48-hr acquisition access period[a]

Inoculation access period	Plants infected/plants tested	
	Chlorotic rosette	Green rosette
	One aphid/plant	
5 min	0/65	0/65
10 min	5/65	5/65
20 min	7/65	6/65
30 min	10/65	8/65
40 min	17/65	12/65
	Five aphids/plant	
6 hr	9/30	8/30
24 hr	17/30	15/30
48 hr	23/30	23/30
Total	88/415	77/415

[a] Data combined from three experiments.

Table 3. Transmission of the viruses causing chlorotic rosette and green rosette diseases to two peanut genotypes by single aphids and groups of two and five aphids (*Aphis craccivora*) after a 48-hr inoculation access period[a]

Aphids per plant	Percent transmission in peanut genotypes		Probability of infection	
	MK 374	F 452.4	Observed	Expected[b]
		Chlorotic rosette		
1	23	20	0.220	0.220
2	27	37	0.320[c]	0.510
5	57	47	0.520[c]	0.832
		Green rosette		
1	30	30	0.300	0.300
2	33	27	0.300[c]	0.510
5	47	43	0.450[c]	0.832

[a] Thirty healthy seedlings were inoculated for each genotype, each disease, and each aphid number.
[b] See text for method of calculation (13,19).
[c] Chi-square values ($P = 0.05$) indicate significant deviation from the expected probability of infection.

access period ranged from 1 to 72 hr and the inoculation access period ranged from 1 min to 3 hr. The retention period was for the lifetime of the vector in all studies. Our results fit within the ranges already reported.

Virus-vector relationships in this study and in past studies (2,6,12,15,18) were limited to one virus in the complex, GRV, because rosette symptoms were used as the criterion for successful transmission and GRAV causes no symptoms (5). Since GRV and GRAV appear to share the same coat protein during aphid transmission (1,10), it seems probable that the acquisition access period, inoculation access period, and latent period are the same for both viruses. However, the vector transmission periods have not been determined for GRAV. Future studies of groundnut rosette should include the determination of GRAV-aphid relationships. Enzyme-linked immunosorbent assays can be used to identify GRAV-infected symptomless plants (1,9).

Results of these studies indicate that the commonly occurring *A. craccivora* in northern Nigeria is capable of transmitting both GRV-C and GRV-G. Furthermore, various aspects of the virus-aphid relationship are similar for the two isolates. Thus, the aphid vector does not appear to be an important factor in the increase in incidence of chlorotic rosette in Nigeria.

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