Stylet Penetration Activities by *Aphis craccivora* (Homoptera: Aphididae) on Plants and Excised Plant Parts of Resistant and Susceptible Cultivars of Cowpea (Leguminosae)

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ABSTRACT Direct current electrical penetration graphs (DC-EPGs) were used to analyze the stylet penetration activities of cowpea aphid, *Aphis craccivora* Koch, on plants of aphid-resistant (ICV-12) and aphid-susceptible (ICV-1) cultivars of cowpea, *Vigna unguiculata* (L.) Walpers. Aphid stylet penetration on whole plants at seedling, flowering, and podding stages were studied in one experiment, and in another experiment excised leaves from seedling plants, excised flowers, and excised pods were tested. Electrical signals depicting the aphid stylet penetration activities on their host plants were amplified, recorded onto a paper chart recorder, and scored for specific waveform patterns. Compared with similar tissues of ICV-1, intact leaves and excised seedling foliage of ICV-12 plants caused severe disruption of aphid stylet penetration activities. This was manifested in frequent penetration attempts that were abruptly terminated or unsustained, and in shorter penetration times, signifying antixenosis resistance in ICV-12. There was reduced occurrence of E waveforms, which represent stylet activity in plant vascular tissues. Also, prior exposure of test aphids to plants of one cultivar did not significantly influence the expected stylet penetration activities on plants of the other cultivar. Overall, ICV-12 exhibited high levels of resistance against *A. craccivora.*

KEY WORDS *Vigna unguiculata*, *Aphis craccivora*, waveform E, electrical penetration graphs, stylet activity, phloem

COWPEA *Vigna unguiculata* (L.) Walpers, is an important staple food crop in Africa and other tropical regions (Singh and Rachie 1985). Cowpea aphid, *Aphis craccivora* Koch, is a serious pest of cowpea that causes extensive crop damage (Jackai and Daoust 1986, Ansari 1984). Infestations often result in significant plant damage including growth deformities, yield reductions, plant mortality, and even crop losses (Singh and Jackai 1985). Damage results from direct physical injury or draining of plant sap during aphid feeding or indirectly through the transmission of plant viruses, including the cowpea aphid-borne mosaic virus (CAbMV) (Bock 1973, Singh and van Emden 1979).

In separate studies of mechanisms of aphid resistance in ICV-12 and other cowpea varieties (Ansari 1984) Givovich et al. (1988) and Firempong (1988) concluded that host plant resistance to *A. craccivora* was governed by both antibiosis (factors deleterious to the aphid life table characteristics) and antixenosis (factors adverse to aphid settling and feeding behavior). However, their characterization of antixenosis in ICV-12 was based mainly on observation of the settling behavior of aphid colonies on plants, and only visual observations of proboscis contact, not actual stylet penetration activities, by individual insects in 'choice' and 'no-choice' tests. Also, those authors did not provide direct evidence of plant tissue localization of aphid resistance factors in cowpea plants. Furthermore, they did not specifically address any implications of plant age or growth stage to the maintenance of aphid resistance, nor did they address the agronomic significance of aphid resistance in specific cowpea varieties that they studied.

Cowpea germplasm used in this work were an aphid-resistant cultivar, 'ICV-12', and an aphid-susceptible cultivar, 'ICV-1', which were registered and released by the International Center of Insect Physiology and Ecology (ICIPE). ICV-1 was a plant selection from landraces in eastern Kenya. ICV-12 was a mutant from ICV-1 generated through γ-irradiation (Pathak and Olela 1986). Both cultivars have similar morphological and phenological attributes, but differ mainly in their resistance or susceptibility to cowpea aphid. Aphid resistance in ICV-12 is monogenic (Pathak 1988).

Electronic monitoring of insect feeding was first described by (McLean and Kinsey 1964), using an

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alternating current (AC) recorder system. Subsequently, Schaefers (1966) reported the use of the first DC-based aphid feeding monitor using a battery as the applied voltage source. Electrical penetration graphs (EPG) based on direct current (DC) were first described by Tjallingii (1978) and represented an improvement over Schaefers' earlier DC monitor. EPGs consist mainly of resistance from conductivity fluctuations through aphid stylets and electromotive forces from voltage sources within live host substrates. DC recorders provide good resolution of signals that can be correlated with biologically significant events, such as the position and activities of stylets in host tissues (Tjallingii 1987, Montllor and Tjallingii 1989, Harrewijn 1990, Tjallingii and Mayoral 1992).

The use of DC-EPGs for analyzing the role of aphid feeding behavior in host plant resistance was recently reviewed by van Helden and Tjallingii (1999) and other aspects of aphid–plant interactions by Annan et al. (1997b), van Helden (1995), Annan (1992), van Helden and Tjallingii (1991, 1993).

DC-EPG signals adopted in this work for analyzing the stylet penetration of *A. craccivora* were a combination of waveform E1, which denotes aphid salivation at initial phases of sieve element puncture, without necessarily resulting in ingestion (Tjallingii 1994, 1995), and waveform E2, which represents sustained passive ingestion in sieve elements (Tjallingii 1990, van Helden and Tjallingii 1991).

Although EPGs (both AC and DC) have been used extensively to study the stylet penetration activities of several aphids on various host plants (Ellsbury et al. 1994, van Helden and Tjallingii 1999), there is only 1 reference to the use of AC-EPGs with *A. craccivora* to document antixenosis in cowpeas (Mesfin et al. 1992). There are no references to any studies of DC-EPGs of *A. craccivora* or other aphid species on cowpeas. In particular, there has been no use of EPG to localize aphid resistance factors in cowpea, as demonstrated with EPGs of *Nasonovia ribis-nigri* on lettuce by van Helden (1995) and van Helden and Tjallingii (1993).

The major objective of this research was to use DC-EPGs to characterize the expression of antixenosis in ICV-12 against the stylet penetration of *A. craccivora*, and thus better classify the resistance in that cultivar, to corroborate the findings by Givovich et al. (1988) and Firempong (1988). Other specific objectives of this work include to identify the plant stages where expression of antixenosis resistance against the aphid is maximum; to determine whether prior exposure of aphids to plants of one cultivar affected subsequent settling and penetration activities on the other cultivar; and to assess effects of earlier plant injury on the expression of ICV-12 resistance.

Materials and Methods

Experimental studies were conducted in the laboratory at the International Center of Insect Physiology and Ecology (ICIPE), Mbita Point Field Station (MPFS) Kenya, in 1990 and 1991. Experiments were conducted in the laboratory at 24 ± 0.50°C, 40–80% RH, and illumination with an overhead panel of 40 W Tezla Z Daylight fluorescent lamps (Tezla, Czech Republic) at an intensity of ≈20 μ $Em^{-2}s^{-1}$.

Aphids. Individuals used in the studies were progenies of a single apterous stem mother that was collected from a cowpea field at the ICIPE-MPFS in February 1990. Colonies were maintained on a susceptible cultivar ('VITA-7') in a greenhouse at 27 ± 1°C, 40–90% RH, and a photoperiod of 14:10 (L:D) h. Apterous adults of similar age (±1 d) were used in tests. Aphids were denied food to increase the likelihood of feeding, and to allow resheathing of their stylets for 1 h before recordings; to prevent dehydration during nonrecording periods, aphids were placed on a piece of moistened filter paper.

Recording System. The EPG recorder unit and methodology used in this study was the same direct current system described in our previous work (Annan et al. 1997a, b). Frequency response of the recording system used was low, although it was sufficient to depict a clear overview of EPG waveforms.

Long durations of recordings are often necessary for plant resistance studies using EPGs to characterize the full array of waveforms produced in the stylet penetration activities of aphids on different plants. However, shorter durations (<1 h) have also been reported (Tjallingii 1986).

Therefore, preliminary tests were done to compare different durations of recording. Those tests indicated that penetrating aphids exhibited most of the EPG waveforms described by Tjallingii (1990) on cowpeas within 1 h of recording time. There were significant differences between ICV-12 and ICV-1 in the incidence and duration of the waveforms. Because we were only interested in comparing early phase host acceptance and settling behavior of *A. craccivora* on the cultivars in this work, the 1-h recording duration was considered adequate, and hence was used for recordings. Also, initial tests of different chart speeds indicated that 40 mm min^{-1} was a reasonable speed to depict representative EPG waveforms, without significantly sacrificing the details of the key features or resolution of observed signals.

Electrical Penetration Graphs Recording on Whole Plants. This experiment was designed to investigate the effects of selected cowpea growth stages on stylet penetration activities of *A. craccivora.*

Test plants were grown in 100-cm^2 Kord Lite pots (Kord, Bramalea, Ontario) with 50–60 g of Black Cotton clay-loam soil. Three-week-old seedling plants, or plants at flowering- and podding stages, were used in the whole plant experiments. On seedling plants, aphids were placed on the abaxial side of leaves, and on flower–stage plants aphids were kept on floral parts, and on podding plants they were restricted to young pods. Aphids were exposed to the different tissues at seedling, flowering, and podding, stages to study their stylet penetration on the tissues that they infest at those respective plant stages in the field.

The experiment was set up using split split-plot design. Two repetitions of experiments were done. The 1st was done between March and April 1990, and

the 2nd was done between November and December 1990. In each repetition there were 2 main plots corresponding to treatments comprising prior exposure of aphids to one cultivar, and then the subsequent switch of those same aphids to the other cultivar. Thus, after recording an aphid on a plant of one cultivar for 1 h, the same insect was then switched to a plant of the other cultivar and recorded for another 1 h. Subplots comprised 3 plant stages (seedling, flowering, or podding), and split subplots consisted of the ICV-12 and ICV-1 cultivars. There were 25 replicates per treatment, and each replicate consisted of a single aphid and single plant combination. Each aphid–plant pair was recorded only once. Incomplete recordings that did not last 1 h were excluded from the data analyses.

Electrical Penetration Graphs Recording on Excised Cowpea Plant Parts. The objective of this experiment was to determine the impact of plant injury or damage (by the excision of tissues) on the expression of ICV-12 resistance against aphid stylet penetration activities.

Four repetitions of the experiment were conducted during the periods of March–April, July–August, and November–December 1990, and in February–March 1991. The experiment was set up as split-plots in a randomized complete block. To avoid confounding the effect of excision of plant parts with prior exposure of aphids to one cowpea cultivar or the other, switching of aphids between cultivars (as was done on whole plants) was not repeated in this study. The main-plot factors were composed of 3 excised cowpea plant parts (leaves, flowers, or pods), which were used as the aphid host substrates. Split-plot factors were composed of the 2 selected cowpea cultivars (ICV-12 and ICV-1). Each treatment combination was replicated 10 times in each repetition. A replicate consisted of a single aphid and plant combination.

Plant tissues that were used as test substrates were excised at their stalks from greenhouse-grown potted plants, and transferred into a 50-ml flask filled with water. To minimize tissue deterioration, recordings were started shortly after excision. Also, each pair of excised tissue and aphid was recorded once.

Response Variables and Statistical Analyses. Data recorded in both experiments included: mean time before the 1st or initial penetration by aphids, mean number of penetrations, total duration of penetration, and duration of waveform E. Scoring of the EPG waveforms was done by measurements from chart paper tracings.

A stylet penetration was defined as any stylet insertion by aphids, regardless of whether the attempted penetration activity reached the phloem tissue (waveform E) or not. Because of the low frequency response of the strip chart recorder, waveforms representing aphid stylet penetration in phloem sieve elements were not strictly differentiated in this study. Therefore, waveforms E1 and E2 were combined to depict general penetration activities of cowpea aphid in cowpea phloem tissues. The combined E1 and E2 patterns were then labeled generally as waveform E.

Distribution of the data collected was tested using Datadesk version 3.0r1 software (Odesta Corporation, Northbrook, IL) (Velleman, 1988). Data for duration of E waveforms did not follow normal distribution, because they ranged widely from very low durations on ICV-12 seedlings to long durations on ICV-1 plants. Therefore, those data were transformed using Log_{10} $(Y + 1)$ (Steel and Torrie 1980). Data were then analyzed for interactions of the split-plot factors using analysis of variance (ANOVA) in SAS/STA software (SAS Institute 1988). Where significant ($P \leq 0.05$) interactions were found, the separate effects of the various experimental factors were determined using multiple comparisons using least significant differences least significant difference (LSD) (Saville 1990). Transformed data were reconverted to the original scale, and results were summarized as means ± SE (SEM).

Results

Nonpenetration and stylet penetration waveforms with potential drops were clearly depicted in the signal tracings. As indicated, waveforms E1 and E2 were combined and labeled generally as waveform E to indicate stylet access to phloem sieve elements. Other waveforms recorded were A, B, C, and F (after Tjallingii 1990).

Waveform E was common on both whole plants and excised plant tissues of ICV-1, and on flowering and podding stage plants of ICV-12. Also, the waveform was observed in recordings on all the excised tissues of ICV-12. However, waveform E was absent or rare on seedling plants of the resistant cultivar ICV-12. The observations of time to the 1st penetration and number of penetrations also confirmed poor aphid settling on ICV-12.

Electrical Penetration Graphs Recording on Whole Plants. Initial inspection of the data collected in the 2 repetitions of the experiment revealed similar trends in effects of the main plot treatments (prior exposure to 1 cultivar or the other), subplots (plant growth stages), and split subplots (crop cultivars) for each parameter (data from ANOVA tables not shown). Consequently, data for both repetitions were pooled before analysis, thus doubling the sample size from 25 to 50 replicates.

Analyses of variance indicated that there were no significant interactions between the main plots (prior exposure of aphids to either cultivar) and subplots (plant growth stage) for time before the 1st penetration ($F = 1.24$; df = 2, 196; $P > 0.10$); number of penetrations ($F = 0.69$; df = 2, 196; $P > 0.10$); penetration time ($F = 1.65$, $0.05 < P > 0.10$; df = 2, 196); and duration of waveform E ($F = 2.06$; df = 2, 196; $P > 0.10$). Also, there were no significant interactions between the main plots and split subplots (cultivar) for time before 1st penetration ($F = 1.68$; df = 1, 294; $P > 0.10$); number of penetrations ($F = 0.30$; df = 1, 294; $P > 0.10$); penetration time ($F = 1.57$; df = 1, 294; $P > 0.10$); and duration of waveform E ($F = 1.48$; df = 1, 294; $P > 0.10$).

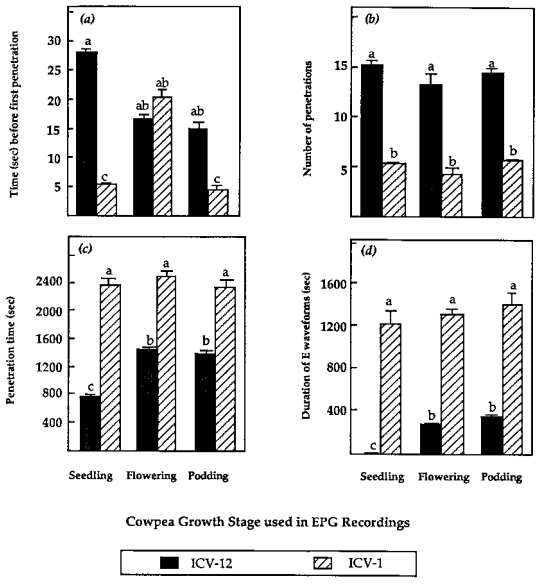

Fig. 1. Cowpea aphid stylet penetration behavior parameters on whole plants of aphid-resistant (ICV-12) and aphid-susceptible (ICV-1) cowpea cultivars at seedling, flowering and podding stages (when test aphids were initially recorded on plants of 1 cultivar for 1 h, and before switching them to plants of the other cultivar). Means (+ SEM) within a column followed by the same letter are not significantly different ($P > 0.05$; LSD test; $n = 50$). (a) LSD = 10.6 for time before 1st penetration; (b) LSD = 8.5 for number of penetrations; (c) LSD = 847.3 for total penetration time; (d) LSD = 626.6 for duration of waveform E.

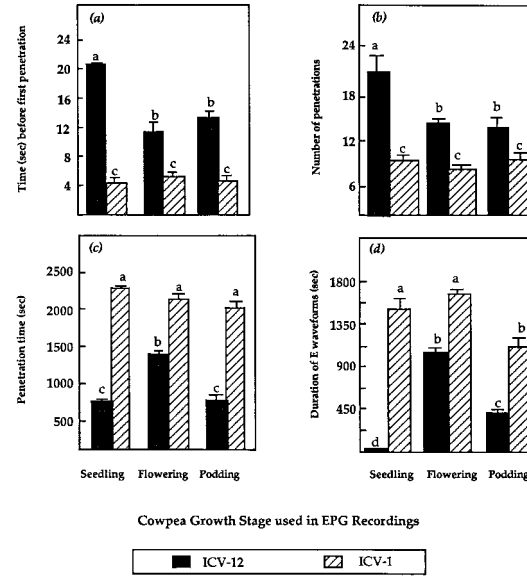

Fig. 2. Cowpea aphid stylet penetration behavior parameters on whole plants of aphid-resistant (ICV-12) and aphid-susceptible (ICV-1) cowpea cultivars at seedling, flowering and podding stages, (when switched and recorded on plants of 1 cultivar for 1 h, after initial recording on plants of the other cultivar for 1 h). Means (+ SEM) within a column followed by the same letter are not significantly different ($P > 0.05$; LSD test; $n = 50$). (a) LSD = 7.7 for time before 1st penetration; (b) LSD = 4.8 for number of penetrations; (c) LSD = 502.9 for total penetration time; (d) LSD = 374.8 for duration of waveform E.

However, the ANOVA indicated that there were significant interactions between crop cultivar selection and plant growth stage tested for the aphid stylet penetration recorded time before the 1st penetration ($F = 56.27$; df = 2, 294; $P < 0.001$); number of penetrations ($F = 37.89$; df = 2, 294; $P < 0.001$); penetration time ($F = 55.37$; df = 2, 294; $P < 0.001$), and duration of waveform E ($F = 23.64$; df = 2, 294; $P < 0.001$).

Although restricted LSD analysis revealed that there were few differences in the responses of the same test aphid to the main plot treatments in the 1st h of recordings (before switching) and the 2nd h of recordings (after switching) (Figs. 1 and 2), the overall ANOVA indicated no significant differences ($P > 0.10$) in the effects of those treatments. However, if the differences indicated by the restricted LSD test were common in all or most of the recorded variables, then we would have considered the main plot treatments biologically significant. But because the overall ANOVA had indicated no significant differences between the main plots, we considered any observed differences to be mere anomalies. Thus, those treatments were considered generally nonsignificant; and we considered that the prior exposure of an aphid to a plant of 1 cowpea cultivar did not affect subsequent stylet penetration of the aphid when it was switched to the other cultivar, as would be expected to occur without the prior exposure treatment to the 1st cultivar. However, to simplify the presentation of the results, means (±SEM) of the crop cultivar selection and plant growth stage of each main plot treatment were summarized separately.

In recordings done both before and after aphids were switched among plants of the 2 cultivars, all growth stages of ICV-12 significantly ($P < 0.01$) reduced the stylet penetration activities of the aphid over ICV-1 (Figs. 1 and 2). The exceptions were in the time before the 1st penetration activity, which did not significantly differ among flowering and podding stages of ICV-12, and the flowering stage of ICV-1 (Fig. 1a); and in duration of waveform E, which was not different between flowering stage of ICV-12 and seedling stage of ICV-1 (Fig. 2d).

Also, analyses by plant growth stage in each cultivar indicated that cowpea aphid penetration on ICV-12 was more adversely affected on seedling plants than on plants at flowering or podding stages (Figs. 1 and 2). However, in recordings made before aphids were switched between test plants of the 2 cultivars, there were no significant differences among the different growth stages of ICV-12 in the number of penetrations (Fig. 1b). In the recordings done after aphids were switched between the cultivars, no significant differences were observed between the seedling and podding stages of ICV-12, but both stages were significantly shorter than the penetration time on flowering

stage plants (Fig. 2c). There were generally no significant differences among growth stages of ICV-1 except for the time to 1st penetration, which was significantly delayed at the flowering stage for recordings made before aphids were switched between plants of the 2 cultivars (Fig. 1a). For recordings made after aphids were switched, the only difference detected among ICV-1 stages was in the duration of waveform E, which was longer in seedling and flowering stage plants than in podding plants (Fig. 2d).

In the recordings made both before and after test aphids were switched among plants of the 2 cultivars, only 3% of aphids (3 of 100 cases) recorded on ICV-12 seedling plants arrived in E waveforms within the total recording periods, albeit only briefly (≈86 s). Also on ICV-12, ≈18% of aphids on plants at flowering stage, and 24% of aphids on podding stage plants could reach phloem sieve elements (waveform E). On ICV-1, ≈49% of penetrating aphids on seedlings, as well as 53% on flowering stage plants and 59% on stage podding plants respectively, exhibited waveform E.

Electrical Penetration Graph Recording on Excised Cowpea Plant Parts. Preliminary observation of the data collected in the 4 separate repetitions of this experiment revealed similar trends in the effects of excised plant part (main plot factor) and cultivar (subplots) (ANOVA data not shown). Thus, the raw data from the 4 repetitions were combined. Hence the total sample size per treatment was 40 replicates.

There were significant interactions between cultivar and plant tissue for all aphid stylet penetration indicators time before the 1st penetration ($F = 8.87$; df = 2, 117; $P < 0.001$); number of penetrations ($F = 44.89$; df = 2, 177; $P < 0.001$); penetration time ($F = 14.36$; df = 2, 117; $P < 0.001$); and duration of waveform E ($F = 4.64$; df = 2, 117; $0.01 < P < 0.025$).

Least significant difference test statistics revealed that, apart from the time before the 1st penetration on excised flowers and excised pods, the other aphid stylet penetration variables were severely reduced on excised tissues of ICV-12 compared with those of ICV-1 (Fig. 3a). No significant differences were detected between the excised flowers and excised pods of ICV-12, or among all excised tissues of ICV-1, in their effects on cowpea aphid stylet penetration activities (Fig. 3). However, compared with excised flowers or excised pods, the excised seedling leaves of ICV-12 produced significant deleterious effects on the aphid stylet penetration.

Approximately 11% of aphids that exhibited penetration on ICV-12 seedling leaves produced waveform E, whereas 34% of aphids on excised flowers and 40% on excised pods exhibited the waveform. On ICV-1, the trends of aphids showing E waveforms were ≈46, 67, and 61% for excised seedling leaves, excised flowers, and excised pods, respectively.

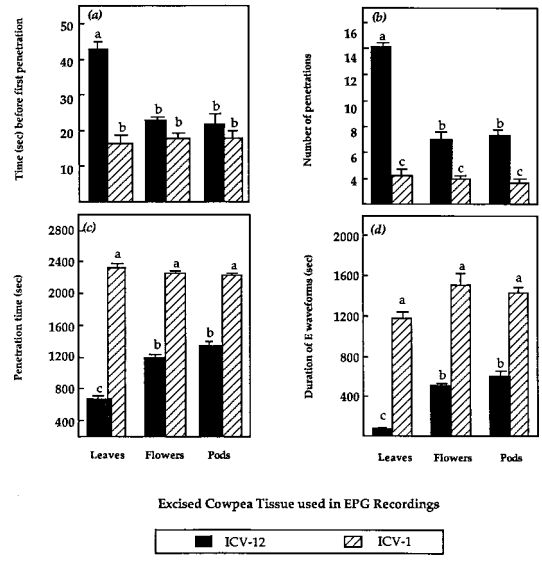

Fig. 3. Cowpea aphid stylet penetration behavior parameters on excised seedling leaves, flowers and pods of plants of aphid-resistant (ICV-12) and aphid-susceptible (ICV-1) cowpea cultivars. Means + SEM bars indicated with the same letter are not significantly different ($P > 0.05$; LSD test; $n = 40$). (a) LSD = 19.8 for time before 1st penetration; (b) LSD = 5.2 for number of penetrations; (c) LSD = 627.5 for total penetration time; (d) LSD = 411.8 for duration of waveform E.

Discussion

Up to 16 h have been reported in similar work on other aphid-plant combinations (Tjallingii 1985, 1986; van Helden 1995). However, it is clear from this work, at least on *A. craccivora*, that 1-h EPG recordings were sufficient to recognize differences in stylet penetration activities on ICV-12 versus ICV-1. Thus, EPG analyses may vary with aphid species, host substrates, or specific interactions between aphids and their hosts. The nature of the particular studies for which the EPGs are being used may also be an important consideration in deciding the duration of recordings. For instance, it may be more reasonable to use shorter EPG recordings to compare aphid stylet penetration on resistant and susceptible crop lines, but longer durations for cataloging general patterns and sequences of the aphid behavior.

The reduction of the stylet penetration by *Aphis craccivora* on whole plants and excised tissues of ICV-12 compared with ICV-1 indicate high levels of aphid resistance in the former, and susceptibility in the latter cultivar. These observations are similar to those reported by MacFoy and Dabrowski (1984). Because plant leaves were tested, it can be deduced that the resistance factor responsible for disrupting aphid feeding behavior was based in or associated with the foliage.

Waveform E was common on all stages and excised tissues of ICV-1, but was reduced on intact ICV-12 plants at flowering and podding, and on excised flowers and pods. The absence of E waveforms in ICV-12 seedlings was indicative of the inability of *A. craccivora* to access the phloem sieve elements, thus indicating a high level of resistance against stylet penetration at

that plant stage. Lack of access to phloem sieve elements suggests that factors that modulate aphid resistance in ICV-12 plants are either based in, or at least associated with, the phloem tissues.

Aphid settling behavior was less successful on ICV-12 than on ICV-1. It took longer for aphids to register 1st penetration on ICV-12 seedling leaves, and they also made several unsuccessful penetration on plants and tissues. The delay in the onset of aphid stylet penetration suggests that plant surface factors are involved in ICV-12 resistance. However, studies of chemical extracts from seedling plants on aphid life table parameters suggested otherwise (Annan et al. 1996). The discrepancy may be explained by the fact that any surface factors that affected stylet penetration activities could have been morphological or physical in nature, but not chemical, and thus were not sequestered in the extracts. Alternatively, if the factor was a chemical, then its effects pertained to aphid feeding but not reproductive behavior.

Compared with seedlings and excised leaves of ICV-12, there was a decrease in the adverse effects of plants and tissues at flowering and podding stages of the same cultivar on the aphid behavior. This apparent decline in aphid resistance in ICV-12 may result from the shift in sink effects from growth and development, into the production of fruiting bodies. The resulting accumulation of sugars and amino acids would stimulate aphids to feed, and thus mask or neutralize any antifeedant factors that would otherwise deter successful penetration.

Effects of excision of plant parts on aphid stylet activities were investigated because, under both field and greenhouse conditions, aphid colonization and population growth was higher on plants of ICV-12 that had been were injured by excision of plant parts, especially at the reproductive phases (flowering, podsetting, and seed-filling) and on senescing plants than on uninjured plants. Such damage ultimately resulted in lowered grain yields. It has been reported that excision of tissues or other forms of injury may cause long-term damage to plants, and consequently alter the expression of resistance against insects (van Emden and Bashford 1976, Tingey 1986, Givovich et al. 1988). Therefore, tissues were cut in this work to simulate natural damage to plants and to assess the impact of injury on the expression of aphid resistance in ICV-12. However, because excision of plant tissues did not significantly alter aphid stylet penetration activities on plants of ICV-12 or ICV-1, the injury from the excision wounds on ICV-12 plants probably does not compromise the expression of aphid resistance in that cultivar.

The study of the effects of prior exposure to 1 cultivar or the other on aphid penetration was undertaken because preliminary observations indicated that when an aphid colony was initially exposed to plants of ICV-1 and then transferred to ICV-12 plants, settling behavior and subsequent population growth were adversely affected. Conversely, in colonies that were 1st exposed to ICV-12 plants and then transferred to ICV-1 plants, population growth was initially slow but later increased sharply. That trend has agronomic significance because in many of the cowpea-growing locations in East Africa, several of those cultivars, other cowpea lines, and other leguminous hosts of *A. craccivora* tend to be planted close to each other in trap cropping, inter-cropping, or other traditional farming systems. There may be important implications for the transmission of plant viruses in cowpeas, because of the negative effect of the resistant cultivars on settling behavior and interplant movement of aphids in the field.

Therefore, our finding that prior exposure of aphids to feeding on 1 cultivar for 1 h did not significantly influence the 1st hour of subsequent stylet penetration activities on the other cultivar is important. This is because although ICV-12 is resistant to the vector (*A. craccivora*), it may not be resistant to stylet-borne nonpersistent or noncirculative plant viruses like CAbMV that are transmitted by the aphid. Because the number of penetrations was high and overall settling was poor on ICV-12, the increased interplant movements and multiple stylet penetration attempts by the aphid on cowpea plants in a field crop might increase significantly. Consequently the transmission of stylet-borne nonpersistent viruses could be high on that cultivar. Thus, crop losses from CAbMV and other aphid-transmitted cowpea viruses could still be significant, although ICV-12 is resistant to the aphid that vectors the disease.

When aphids were switched between plants of the 2 cultivars (for example, 1st recorded on ICV-12 plants for 1 h then switched and recorded for another 1 h on ICV-1 plants, or vice versa), prior exposure of the aphids to plants of 1 cultivar did not significantly affect their subsequent stylet penetration activities on plants of the other cultivar. Anecdotal observations by Annan et al. (1997a) indicated that aphids that had been previously exposed to the susceptible cultivar (ICV-1) could not colonize or reproduce successfully on plants of the resistant cultivar (ICV-12). This indicated a strong impairment of aphid settling behavior on the latter cultivar. Also, aphids that had prior exposure to ICV-12 could not successfully settle on ICV-1 initially, although they were eventually successful in colonizing the susceptible cultivar. This suggests that there was a residual or delayed effect of the exposure of aphids to ICV-12 on their ability to settle and colonize plants of even susceptible cultivars. Nevertheless, the affected aphids eventually recovered from the effects of the cultivar after they were removed. Thus, ICV-12 manifested short-term adverse effects against aphid behavior and biology even after individuals had been removed from plants of that cultivar, and transferred to plants of the susceptible cultivar.

Annan et al. (1997a) reported that wiring of aphids and other treatments for EPG recordings did not significantly affect aphid stylet penetration. However, cowpea cultivar selection significantly affected stylet activities. So, it is reasonable to conclude that the differences observed in this work did not result merely from artifacts of the treatments, or methods required for EPG recordings. Thus, the use of EPGs in this work

provides a valid bioassay technique for evaluating the influence of host plant resistance in ICV-12 on settling behavior and the stylet penetration activities of *A. craccivora.*

Overall, our results here indicate that ICV-12 caused severe adverse effects on cowpea aphid stylet penetration. This suggests that antixenosis or nonpreference (Painter 1951) is a category of aphid resistance in ICV-12. Evidence reported elsewhere (Annan et al. 1992, 1996, 1997b, c) suggests that ICV-12 also shows antibiosis against *A. craccivora.* Thus, the cultivar exhibits a dual mechanism of aphid resistance. This assertion corroborates similar findings made by Firempong (1988) and Givovich et al. (1988).

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