# The Citrus Exocortis Disease: A Complex of Viroid-RNAs

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ABSTRACT. Citrons inoculated with different field sources, displayed a variety of symptoms ranging from very mild leaf bending and necrosis to the severe reaction normally associated with exocortis disease. Nucleic acid preparations from shoot samples were analyzed by sequential polyacrylamide gel electrophoresis. All source from both California and Spain contained one to four viroids with distinct physical and biological properties. The size range was estimated from 371 nucleotides for the citrus exocortis viroid (CEV) to 275 for the smallest viroid.

The recovery of single viroids suggested a relationship between the distinct viroids and the symptom reaction expressed in citron.

*Index words.* Citron variable viroid, citrus viroid complex, electrophoresis, nucleic acids, hybridization assays.

Since the first description of the exocortis disease by Fawcett and Klotz (5), degrees of stunting, differences in mode of formation of bark scaling and time of appearance of symptoms on sensitive rootstocks such as trifoliate orange have been reported. These were attributed to different strains of the disease agent (2, 3, 7, 8 , 9, 14, 17). With the transmission of the disease to citron, and the selection of sensitive citron clones such as USDCS 60-13, Arizona 861 and Arizona 861-S1 (1, 16) as indicators, more sensitivity and faster exocortis indexing was achieved. In the process, the use of trifoliate orange as the indexing host for the exocortis disease was virtually abandoned. Therefore, in most cases the bark shelling reaction in trifoliate orange has not been reproduced with exocortis disease isolates identified by the citron reaction.

Browning on the tips of the leaf blades, petiole wrinkle and necrosis, midvein necrosis, leaf epinasty and stunting are among the symptoms observed on inoculated Arizona 861-S1 citrons. All these symptoms were considered as diagnostic evidence of mild, moderate or severe strains of exocortis when detected during indexing of symptomless stock/scion combinations.

The transmission of severe forms of the disease to *Gynura aurantiaca* D.C. and other herbaceous hosts (30), permitted the isolation and characterization of the causal agent as a low molecular weight, infectious RNA, the citrus exocortis viroid (CEV) (21, 22). Citrons inoculated with purified CEV preparations produced severe epinasty, leaf rugosity and stunting. Further biochemical and biophysical characterization of the causal agent of the exocortis disease (6, 20, 23, 24, 29) has focused on viroid RNA from *G. aurantiaca* D.C., tomato and *Chrysanthemum morifolium* Ram. which included, in addition to the 371 nucleotide viroid (29) some variants with minor differences in the number of nucleotides residues ranging from 370 to 375 (27, 28).

Isolates producing mild and moderate reactions on citron did not cause symptoms in *Gynura*. These early observations suggested either the existence of many forms of "exocortis disease", undetectable levels of the agent, or *Gynura* as a selective host.

Recently, the "citron variable viroid (CVaV)" isolate (18), now known to comprise a complex of four viroids with distinct electrophoretic mobilities have been described along with other viroid species and shown to be associated with citron plants dis-

playing a variety of symptoms ranging from mild to moderate in citron (4). Since the symptom expression of the CVaV isolate has not been associated with any one or more of the four viroid components, the name "citron variable viroid" presents a misleading terminology.

This paper presents additional information describing different viroid RNAs associated with the broad "exocortis disease" reactions on citron, and a provisional grouping of these citrus viroids (CV) is proposed based on their physical and biological properties.

## VIROID PROFILES OF CITRON ISOLATES

**Source materials.** The citrus viroids described here were derived from inoculation of Etrog citron (Arizona 861-S1) with various field sources. The stable citron isolates constitute either single viroids or mixtures of 2-4 viroids. In many cases the original field sources are no longer available.

**Polyacrylamide gel electrophoresis.** Nucleic acid preparations were obtained from shoots of citrons inoculated with different field sources by phenol extraction and lithium chloride partitioning (4, 24). When analyzed by sequential gel electrophoresis under native and denaturing conditions (4, 20) followed by silver staining (10), one or more viroid RNAs were detected in each sample (4). In a previous report (4), an attempt to classify the different field isolates on the basis of their viroid profiles was presented. However, a new technique, which enhances the resolution of circular viroid molecules

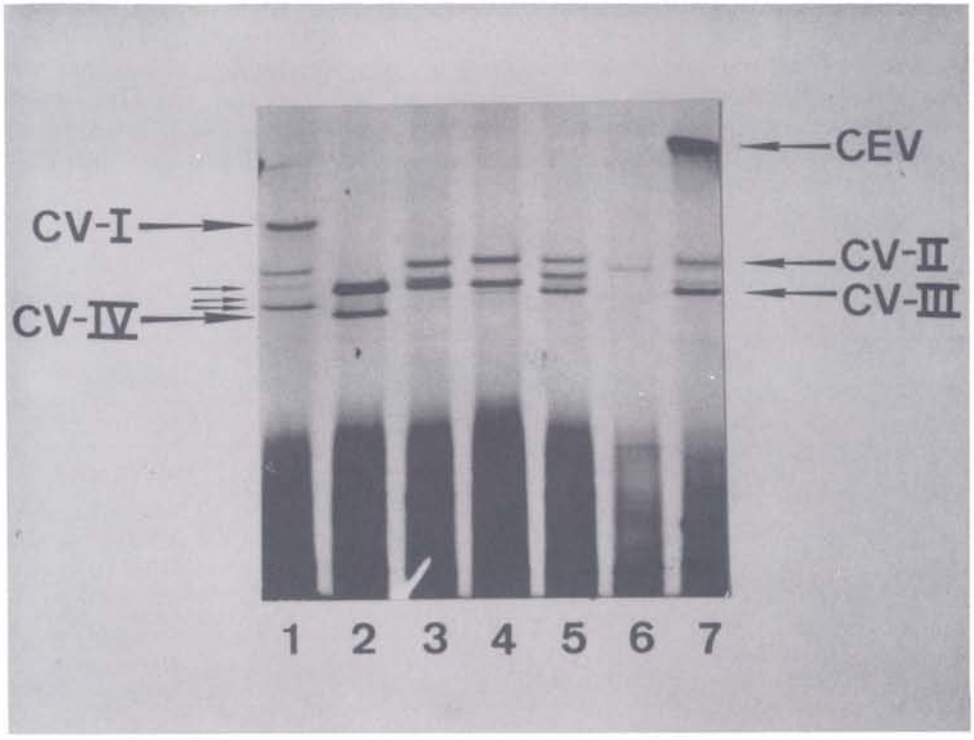



**Fig. 1. Polyacrylamide gel electrophoresis of nucleic acid extracts under low pH, denaturing conditions (8M urea) following electrophoresis in 5% gels. Samples were from isolates E144, E152, E151 and E124 from Spain (lanes 1, 3, 5 and 7), and isolates E804, a mixture of E818 and E805 and purified CV-IIb from California (lanes, 2, 4 and 6). Small arrows indicate the migration region of CV-IIIs. Gels were silver stained.**

(13), revealed an almost continuous array of viroid RNAs with different electrophoretic mobilities in the area of CV-II and CV-III (fig. 1) whereas only these two distinct bands had been recognized earlier.

The relative mobilities of the viroids found in selected sources from California and Spain were compared by co-electrophoresis and a consensus catalogue of citrus viroids was established. Table 1 describes five major groups of citrus viroids defined by similar electrophoretic mobilities. This was estimated by comparison of their relative migration in denaturing PAGE with the citrus exocortis viroid (CEV) and the avocado sunblotch viroid (ASV). As shown later, the components of each group also display similar physical and biological properties.

**Viroid profiles of "exocortis" isolates found in California and Spain.** A series of stable "exocortis" isolates (judged by symptoms on inoculated citron plants) were recovered from different citrus sources. These isolates produced a variety of reactions in citron ranging from very mild leaf necrosis, to petiole wrinkle and necrosis, midvein necrosis, leaf curling and epinasty, and different degrees of stunting. Analysis by sequential gel electrophoresis revealed characteristic viroid profiles associated with individual isolates (table 2).

Spanish sources in general, contain more viroids than sources from California. This is probably a result of topworking, a common practice in Spain. Top worked trees would accumulate all viroids from the different budwood sources into a single tree. In contrast, single viroids were found in sources from California where topworking is not a common practice.

Some viroids such as CEV, CV-IIa, and CV-IIIb are widespread in both California and Spain, whereas CV-Ib, CV-IIIa and CV-IV are restricted to a few sources from California. Another viroid, CV-IIb, found only as a component of E821, is the causal agent of the cachexia disese, and the main viroid component of the cachexia isolates (25). Two other viroids, CV-IIIc and CV-IIId, were widespread in the Spanish collection, but were not found in California sources.

TABLE 1
PHYSICAL PROPERTIES OF CITRUS VIROIDS (CV)

| Citrus viroids | Bases[z] | CF-11 Cellulose[y] Ethanol 25% | CF-11 Cellulose[y] Elution 20% | cDNA[x] hybridization CEV | cDNA[x] hybridization CV-Ib | cDNA[x] hybridization ASBV |
|---|---|---|---|---|---|---|
| CEV | 371 | — | + | ++++ | ± | — |
| CV-Ia | 340 | — | + | — | ++++ | — |
| CV-Ib | — | — | + | — | ++++ | — |
| CV-IIa | — | + | — | — | — | — |
| CV-IIb (CCaV) | 300 | + | — | — | — | — |
| CV-IIIa | — | + | — | — | — | — |
| CV-IIIb | 290 | — | + | — | — | — |
| CV-IIIc | — | NT[w] | | — | — | — |
| CV-IIId | — | NT | | — | — | — |
| CV-IV | 275 | + | — | + | — | — |

[z]By comparison with Citrus Exocortis Viroid (CEV) and Avocado Sunblotch Viroid (ASV) in denaturing PAGE.
[y]CF-11 ellulose elution indicates ethanol % to begin specific viroid elution.
[x]Relative reaction with cDNA as determined by slot-blot and electroblot hybridization.
[w]NT = not tested.

TABLE 2
DISTRIBUTION OF CITRUS VIROIDS IN STABLE ISOLATES FROM CALIFORNIA AND SPAIN

| Isolate | Citron Reaction[z] | Viroid content | | | | | | | | | | Duplicate[y] Isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CEV | CV-Ia | CV-Ib | CV-IIa | CV-IIb | CV-IIIa | CV-IIIb | CV-IIIc | CV-IIId | CV-IV | |
| California: | | | | | | | | | | | | |
| E 811 | Var. | + | — | — | — | — | — | — | — | — | — | |
| E 820 | 5 | + | + | — | — | — | — | — | — | — | — | |
| E 814 | 5 | + | — | — | + | — | — | + | — | — | — | 800,809 |
| E 812 | 5 | + | — | — | — | — | — | + | — | — | — | |
| E 803 | 2 | — | + | — | + | — | — | + | — | — | — | |
| E 821 | 3 | — | — | + | + | + | — | + | — | — | — | |
| E 818 | 1 | — | — | — | + | — | — | — | — | — | — | 819 |
| E 822 | 3 | — | — | — | — | — | + | — | — | — | — | |
| E 805 | 2 | — | — | — | — | — | — | + | — | — | — | 806,807 |
| E 804 | 4 | — | — | — | — | — | + | — | — | — | + | |
| Spain: | | | | | | | | | | | | |
| E 161 | 4 | + | + | — | + | — | — | — | + | + | — | 123 |
| E 117 | 4 | + | + | — | + | — | — | — | — | + | — | 160 |
| E 124 | 4 | + | — | — | + | — | — | — | + | + | — | |
| E 104 | 4 | + | — | — | + | — | — | + | — | — | — | 128,100 |
| E 129 | 3-4 | + | — | — | + | — | — | — | + | — | — | 120,132,106 |
| E 144 | 2-3 | — | + | — | + | — | — | + | — | + | — | |
| E 151 | 2 | — | — | — | + | — | — | + | — | + | — | |
| E 107 | 1-2 | — | — | — | + | — | — | — | + | — | — | 122,119 |
| E 152 | 1 | — | — | — | + | — | — | + | + | — | — | |
| E 111 | 1 | — | — | — | + | — | — | + | — | — | — | 125,127,143 |

[z]Indexing was performed on Arizona 861-S1 as described in Agricultural Handbook 333, (1). The citron reaction was rated on a 0-5 scale for California isolates and a 0-4 scale for Spanish isolates with 0 = healthy and 4 or 5 = severe.
[y]Isolates with same profile as isolate listed on left.

## PHYSICAL PROPERTIES

**Cellulose affinity.** The present knowledge about the differential binding properties of viroids to cellulose (19) indicates that different viroids begin elution at distinct ethanol concentrations when performing cellulose chromatography. The elution properties of citrus viroids in CF-11 cellulose chromatography are summarized in table 1. These properties can be utilized as a discriminating tool for the recovery of preparations enriched for specific viroids, or as an additional parameter for viroid characterization.

When nucleic acid-cellulose preparations containing CV-IIa (RNA-II) were extensively washed with buffer containing 25% ethanol, as previously reported (4), this viroid was not recovered from the final eluant. However, we have been able to obtain CV-IIa-enriched preparations by washing the cellulose columns first with buffer containing 30% ethanol and eluting with 25% ethanol buffer and—or buffer alone. Analysis of the enriched preparations provided evidence for the viroid nature of RNA-II (Duran-Vila *et al.*, unpublished). In addition, the utilization of CF-11 cellulose chromatography permitted the discrimination of CV-IIIa and CV-IIIb even though these have very close electrophoretic mobilities.

**Sequence homology based on hybridization with other viroids.** Extracts from tissue infected with selected isolates (LiCl supernatant samples or CF11 chromatographed preparations) were denatured (31), applied to nitrocellulose membranes using an Hibri-slot filtration manifold (BRL), and hybridized to cDNA made to CEV and CV-Ib, as well as to ASV (a non-citrus viroid control). The cDNA was synthesized by the method of Taylor *et al.*, (26). The reactions were dependent on the viroid profile of the isolate and the cDNA probe utilized. As summarized in table 1, cDNA to CEV-RNA showed a strong hybridization reaction only with preparations from isolates containing CEV (fig. 2). A weak reaction was also detected with preparations from source E804 which did not contain CEV, but contained CV-IIIa and CV-IV. Hybridization of an electroblot from an E804 extract on denaturing PAGE gave a weak positive reaction with CV-IV and no reation with CV-IIIa.

A positive reaction was also observed when samples containing CV-Ia or CV-Ib were hybridized with cDNA to CV-Ib. An electroblot procedure was also utilized to confirm the homology of CV-Ia and CV-Ib. In this case, however, the reaction was very strong, indicating a high degree of homology of CV-Ib for CV-Ia. It is interesting to note that CV-Ib is restricted to California isolates whereas CV-Ia occurs in both California and Spain. Thus, perhaps the smaller CV-Ib may comprise a recent deletion derivation of CV-Ia in California. No hybridization was detected with any of the samples tested to cDNA to ASV.

Although these hybridization results do not provide a complete picture of the sequence homology among citrus viroids, they support the segregation of citrus viroids into five separate groups. Additional hybridization tests using probes made to a CV-III viroid, and, ultimately, base sequencing is needed to define sequence relationships among the citrus viroids.

## BIOLOGICAL PROPERTIES

**Transmission to herbaceous hosts.** Extracts from selected isolates derived from field sources were slash inoculated to *Gynura aurantiaca* DC, tomato, eggplant, *Gomphrena globosa* L. and *Datura stramonium* L.. *Gynura* and tomato plants inoculated with CEV-containing isolates showed stunting, epinasty and leaf rugosity characteristic of CEV infection 3 to 4 weeks after inoculation, whereas the other species were symptomless. Inoculation with isolates which did not contain CEV, did not induce

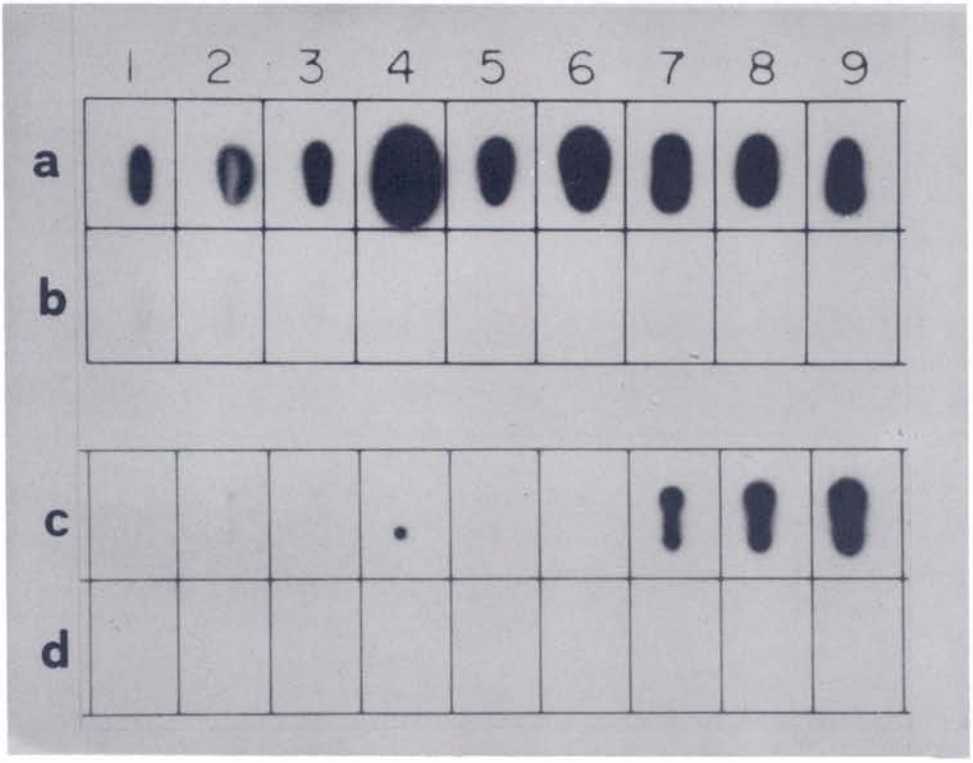



**Fig. 2. Autoradiographs of slot-blot hybridizations on nitrocellulose membranes of nucleic acid preparations of citron and Gynura inoculated with different exocortis isolates using a cDNA to citrus exocortis viroid (CEV) probe. In all instances 10 µl samples were applied using a Hybri-slot filtration manifold. a) Samples were 2M LiCl soluble nucleic acids of CEV containing isolates: E117 and E129 on Gynura (1 and 2), and E117, E123, E104, E129, E124 and E128 on citron (3 to 9). b) Samples were 2M LiCl soluble nucleic acid preparations of mild isolates: E125, E122, E147, E146, E152, E151, E153 (1 to 7), pure CV-Ia (8) and CV-IIa (9) on citron. c-d) Samples were nucleic acid preparations after CF-11 cellulose chromatography of healthy (1) and isolates E807, E804, E819, E821 , E803, E820, E812, E814 (2 to 9) on citron. Eluants at 25% EtOH (d) and D0% EtOH (c) of the same preparations were processed simultaneously.**

symptoms in any of the five species. All inoculated plants were pruned four weeks after inoculation. The second growth flush of tissue was collected and the nucleic acids extracted as before and analyzed by sequential gel electrophoresis. A CEV band was observed in all hosts whether symptomatic and symptomless, whereas CV-II was detected only in eggplant (fig. 3). The CV-II-containing eggplants were symptomless.

Cucumber seedlings of the variety 'Suyo' (kindly provided by Sakata Seed Corp.) were inoculated by stem puncture when the first true leaves were just emerging. All inoculated cucumbers were symptomless and were pruned three weeks after inoculation. When the second growth flush emerged characteristic symptoms were observed on plants inoculated with CV-II-containing sources. Both CV-IIa and CV-IIb (25) induced darker color, shorter internodes, smaller leaves with rugose leaf blades and distorted fruits. When the nucleic acids were extracted and analyzed by sequential gel electrophoresis CV-IIa and CV-IIb were detected from symptomatic plants. A CEV band could also be detected from symptomless plants which had been inoculated with CEV-containing isolates (fig. 3).

The infectivity of CV-IV was tested only on Gynura and cucumber.

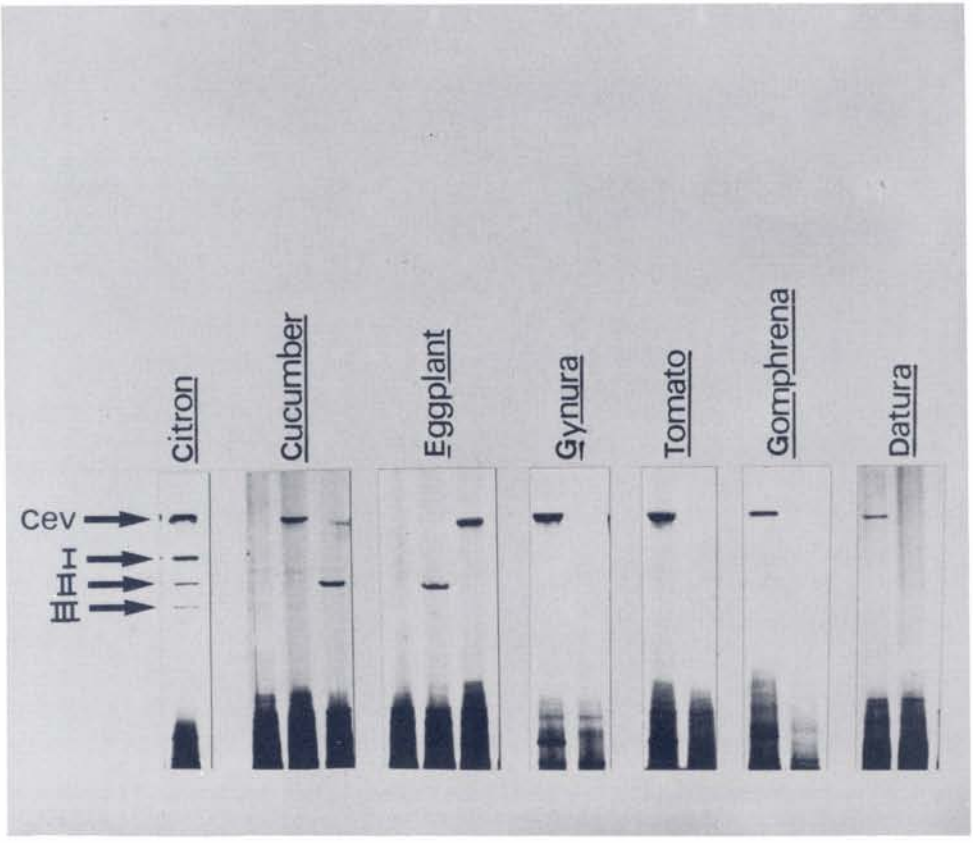



**Fig. 3. Polyacrylamide gel electrophoresis (PAGE) of nucleic acid extracts under low pH, denaturing conditions (8M urea) following electrophoresis in 5% gels as described as sequential PAGE. Samples were from healthy and inoculated cucumber, eggplant, gynura, tomato, Gomphrena and Datura. An electrophoretic profile of isolate E117 from citron is shown as a reference for migration of the different citrus viroids. Gels were silver stained.**

This viroid was recovered from symptomless inoculated cucumber plants (table 3).

Therefore, in addition to the described differences in physical properties, citrus viroids have quite different host ranges. Whereas CEV can be recovered from a large number of herbaceous hosts (30) and induces characteristic symptoms in several of them, CV-II and CV-IV have a more restricted host range. No host outside the Rutaceae has yet been found for CV-I and CV-III. Therefore, citron remains the common host from which all described citrus viroids can be easily recovered.

**Symptomatology in citron.** Although citrus viroids are usually found as mixtures, single viroid infections have been produced by inoculation with nucleic acid preparations from herbaceous hosts (CEV, CV-II and CV-IV) or by electroelution of single bands obtained after performing sequential PAGE (CV-I and CV-III). Single viroid sources have also been obtained by shoot tip grafting *in vitro* (see next section).

Inoculations of citrons with pure CEV preparations caused severe stunting, leaf epinasty and necrosis (fig. 4A and 4B). Differences in symptom intensity have been found among different samples and are thought to be caused by minor differences in the nucleotide sequence and in the number of nucleotide residues, as described by Visvader and Symons (28). Citron plants inoculated with

TABLE 3
BIOLOGICAL PROPERTIES OF CITRUS VIROIDS

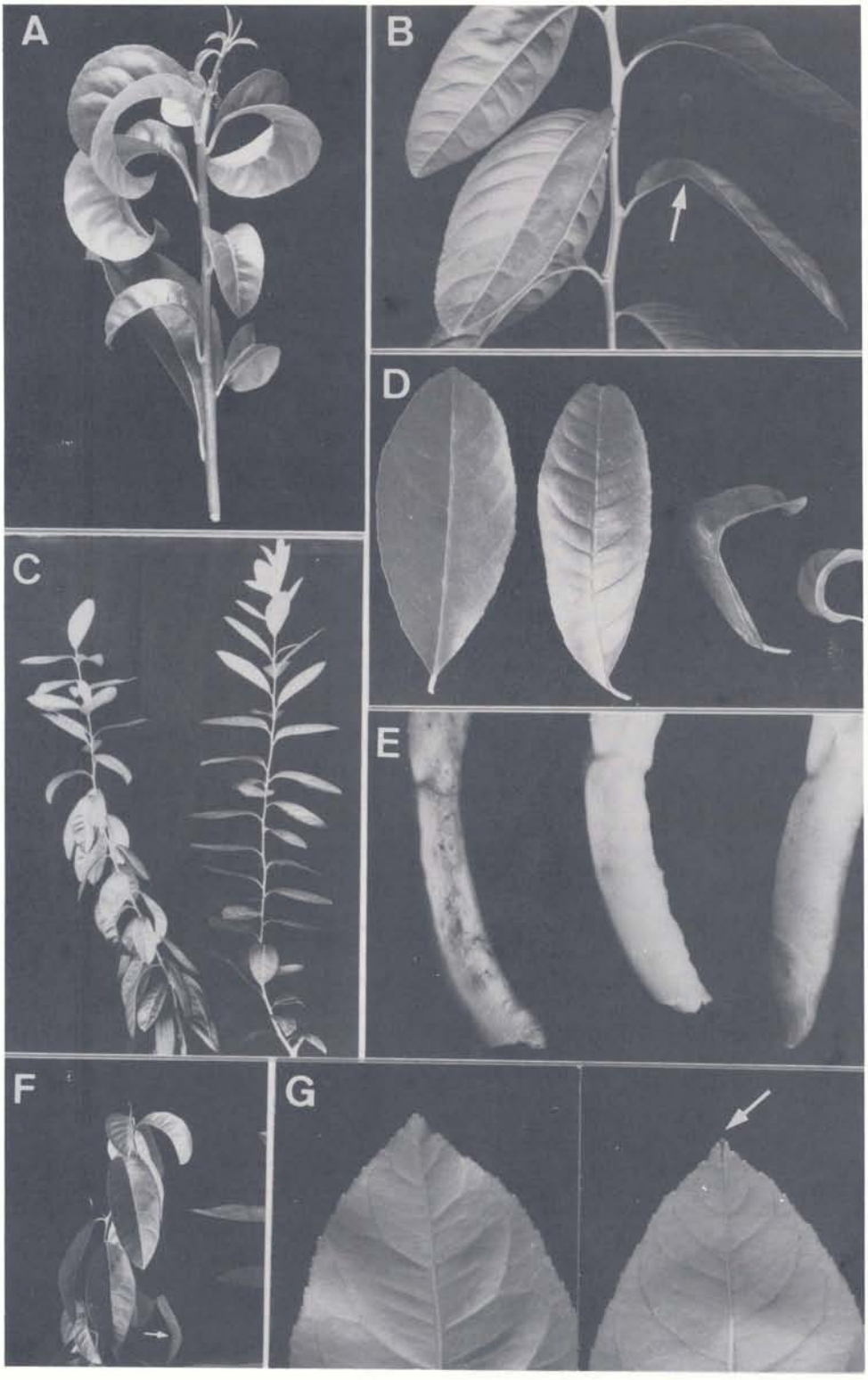
| Citrus viroids | Infectivity | | | Symptoms in Citron | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gynura | Cucumber | Citron | stunting | midvein necrosis | leaf epinasty | petiole necrosis | leaf tip browning |
| CEV | + | + | + | severe | general | severe | general | ? |
| CV-Ia | — | — | + | ± | local | random | — | — |
| CV-IIb | — | — | + | ± | local | random | — | — |
| CV-IIa | — | + | + | none | — | — | — | faint |
| CV-IIb | — | + | + | none | — | — | — | — |
| CV-IIIa | — | — | + | ± | local | random | — | — |
| CV-IIIb | — | — | + | ± | local | random | — | — |
| CV-IIIc | — | — | + | ± | local | general | — | — |
| CV-IIId | — | — | + | mild | general | general | — | |
| CV-IV | — | + | + | ± | local | random | — | — |

preparations containing CV-Ia or CV-Ib showed a pronounced epinasty or bending of the leaves because of point necrosis of the midrib on the underside of the leaf blade (fig. 4B and 4D). This symptom occurs in a random fashion and affects only a few leaves of the inoculated plants. In general, citrons inoculated with pure sources of CV-IIa or CV-IIb were symptomless. However, E818 and E819, which are naturally occurring pure sources of CVC-IIa, produced a mild response when inoculated on Arizona 861-S1 citron under optimum conditions of temperature and day length. The reaction was a faint necrosis of the tip of the leaf blade (fig. 4G) in a small number of leaves and sometimes a very mild petiole wrinkle and occasional petiole browning. All sources of CV-IIb failed to show symptoms on citrons incubated under a range of environmental conditions.

The symptoms induced by CV-III were characterized by different degrees of petiole wrinkle and necrosis (fig. 4E) accompanied by midvein necrosis which sometimes affected secondary veins. This resulted in a marked leaf epinasty which was general (fig. 4C), or randomly affected only some leaves (fig. 4F). Some degrees of mild stunting also developed. A comparative study to determine whether the different CV-III group viroids can be discriminated by symptomatology on citrons has not yet been made. Inoculation of citrons with pure CV-IV resulted in random leaf epinasty and midvein necrosis (table 3).

Comparison of the symptoms in citron produced by single viroids with those produced by mixed infections indicates existence of synergistic viroid interactions. The E821 isolate, originally described as producing moderately severe symptoms, under optimal environmental conditions caused symptoms in citron similar to those induced by CEV, even though CEV is not a component of this complex isolate. Under different environmental conditions (18), a variable reaction with periodic production of symptomless and symptom-expressing growth was evident. These symptoms were more severe than expected from a combination of the symptoms induced by each component poresent. Also, the variable nature of the citron response suggest that different components of the viroid complement predominate under different environmental conditions. Similarly, the mild symptoms associated with both CV-IIIa and CV-IV look unrelated to the moderate symptoms induced by the original isolated E804 which contains both viroids.

**Artificial viroid mixtures.** The majority of the "exocortis" isolates


A
B
C
D
E
F
G

studied were found to occur as viroid mixtures (table 2). Although the number of isolates described does not include all possible viroid combinations, artificial mixtures have been obtained and also shown to be stable.

In fact, the recovery of stable, mild forms of "exocortis" by knife blade transmission from severely affected citron donors was already reported in 1969 (15). Although the process was not understood at that time, the possibility of several strains of the disease existing as components of a disease complex was suggested. Isolates E806, E811 and E812 from California were recovered by knife transmission from a single donor (E800). These were originated by independent transmission of the viroid components present in the original source which resulted in two single viroid isolates and one isolate containing two viroids (table 2).

Other artificial viroid profiles have also been produced by greenhouse and laboratory manipulations (table 4). The differences in transmission properties of the different viroids can be utilized to segregate some of the components present in a complex isolate. In fact, *Gynura* and tomato, which have long been utilized as primary hosts for viroid purification, also functioned as selective hosts for CEV. The availability of cucumber and eggplant as screening hosts for CV-II should help in the identification and characterization of members of this viroid group.

The application of shoot-tip grafting *in vitro* (12) to Arizona 861-S1 citrons infected with the Spanish isolate E117 also resulted in two single viroid sources and a source containing a combination of two viroids (table 4).

The production of stable combinations and single viroids suggests that all the viroids tested here can be obtained as pure isolates and all viroid combinations should be possible. In fact, attempts to obtain viroid combinations by mixed inoculations have been successful (table 4).

## HORTICULTURAL IMPLICATIONS

During the early studies on the transmission of the exocortis disease, severe stunting, epinasty and leaf rugosity induced on inoculated citrons were associated with severe dwarfing and bark scaling observed in field trees grown on sensitive rootstocks (2, 5, 14). Also, a large number of mild and moderate sources identified according to their reaction on citron were detected during routine indexing of symptomless field trees grown on sour orange and other symptomless rootstocks. Therefore, the precise relationship between the citron reaction and the overall disease syndrome in the field remains unclear.

With this present understanding, it appears that the "exocortis disease", as has been developed in the literature, constitutes a complex of apparently unrelated viroids, some of which may not produce the classical symptom of the disease on trifoliate orange. In addition, the availability of pure viroid sources will provide a better understanding of the described "dwarfing factors". Perhaps a viroid responsible for dwarfing alone could be safely separated from deleterious mixtures or engineered from laboratory produced mixtures.

**Fig. 4. Symptomatology in citron after inoculation with CEV and citrus viroids (CV). A) Severe stunting and epinsaty induced by pure CEV. B) Bending of the leaves as a result of point necrosis of the midrib on the underside of the leaf blade (see arrow) induced by pure CV-Ia. C) Leaf epinasty as a result of petiole bending induced by pure CV-IIIc. D) Leaf symptoms induced by pure CEV (right), pure CV-Ia (middle right), pure CV-IIIc (middle left) and healthy control (left). E) Healthy control (right), and petiole wrinkle (middle) and necrosis (left) induced by pure CV-IIIc. F) Symptoms induced as a result of a mixed inoculation (CV-Ia, CV-IIa and CV-IIId). G) Faint necrosis of the top of the leaf blade (see arrow) induced by CV-IIa (right) and healthy control (left).**

TABLE 4
VIROID PROFILES OBTAINED THROUGH LABORATORY AND GREENHOUSE MANIPULATION

| Technique | Isolate origin | Viroid Profile | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CEV | Ia | Ib | IIa | IIb | IIIa | IIIb | IIIc | IIId | IV |
| Screening host: | | | | | | | | | | | |
| Gynura | "severe"[z] | + | — | — | — | — | — | — | — | — | — |
| Cucumber | E 144 | — | — | — | + | — | — | — | — | — | — |
| Cucumber | E 821 | — | — | — | + | + | — | — | — | — | — |
| Cucumber | E 804 | — | — | — | — | — | — | — | — | — | + |
| Selective removal: | | | | | | | | | | | |
| Shoot tip grafting | E 117 | — | + | — | — | — | — | — | — | — | — |
| " | E 117 | — | — | — | — | — | — | — | — | + | — |
| " | E 117 | — | + | — | — | — | — | — | — | + | — |
| " | E 117 | — | + | — | + | — | — | — | — | — | — |
| Selective transmission: | | | | | | | | | | | |
| Inoculation | E 117 | + | — | — | + | — | — | — | — | — | — |
| " | E 117 | — | + | — | + | — | — | — | — | — | — |
| " | E 117 | + | + | — | + | — | — | — | — | — | — |
| " | E 117 | + | — | — | + | — | — | — | — | + | — |
| " | E 117 | — | + | — | + | — | — | — | — | + | — |

[z]Citrus exocortis viroid (CEV) can be recovered from Gynura inoculated with all "severe" isolates.

In order to define the cause-effect relationship between the different citrus viroids and the "exocortis disease", it will be necessary to study the performance of trees inoculated with single viroid and specific viroid combinations. The results of this testing will identify the potential disease causing agents and their relationship to the exocortis disease as originally defined.

Some preliminary results are already available from inoculations made in 1977 and 1983 with field isolates from California. These results illustrate the significant stunting effect of CEV on trees on Troyer citrange as well as on sour orange whereas the other citrus viroids had little effect on trees grown on the same rootstocks (11). However, sweet orange trees grown on trifoliate orange which were inoculated with E818 and E819 (naturally occurring pure sources of CV-IIa) in 1983 were already showing the first symptoms of bark cracking. Therefore, CV-IIa the viroid causing the mildest symptoms on citron induced the classical symptoms associated with the exocortis disease in the field. The further development and persistence of this reaction in the field trees must be evaluated.

A citron reaction to CV-IIa has not been consistently observed. In many instances citrons inoculated with CV-IIa were symptomless, and an Arizona 861-S1 citron line maintained at IVIA as a healthy source was found to carry CV-IIa. Therefore, one should exercise caution when using citron as the sole indicator for citrus viroids.

## CONCLUSIONS

The data presented here on the role of several viroids on the development of symptoms in citron, which were originally thought to be associated with the exocortis disease, provides the basis for a new understanding of the disease and its causal agents.

The study of a number of naturally occurring field isolates, and the determination of the physical and biological properties of their viroid components permits the cataloguing of citrus viroids into five different groups. Although ten viroids from California and Spain, with different elec-

trophoretic mobilities have been used as the basis for a consensus catalogue of citrus viroids, it is likely that the analysis of other isolates in different citrus growing areas of the world will result in further elaboration of this grouping. Studies of the physical and biological properties of these isolates will probably reveal the existence of and even larger number of viroids with distinct properties.

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