

## ABSTRACTS

### Defective RNA Molecules Associated with Citrus Tristeza Virus

M. Mawassi, A. V. Karasev, E. Mietkiewska, R. Gafny, R. F. Lee, W. O. Dawson, and M. Bar-Joseph

ABSTRACT. Single-stranded (ss) RNA extracted from citrus tristeza virus (CTV) particles of the Israeli VT strain and double-stranded (ds) RNA preparations extracted from infected plants, contained a population of molecules with features that suggest that they are defective (D) RNAs. The prototype of 2,424 nt was found to be composed of the genomic RNA termini of CTV. Recently, two other D-RNA of 2.7 and 4.5 kb with a similar type of termini but with different junction sites were isolated from CTV-VT-infected plants. Northern hybridization analyses demonstrated that the D-RNAs were normally more abundant than other CTV-specific ss- and ds-subgenomic RNAs in infected plants. All of the three presently characterized D-RNAs were found encapsidated by the CTV coat protein. The possible involvement of D-RNAs in the biology of CTV is discussed.

### Comparison of a p27 Gene Fragment Among Biologically Diverse Spanish Isolates of Citrus Tristeza Virus

S. Gago-Zachert, R. A. Dewey, P. Moreno, L. Semorile, and O. Grau

ABSTRACT. Previous work showed that a fragment of a clone of the citrus tristeza virus (CTV) genome hybridized differentially between a mild (T312) and a severe (T387) CTV isolate. Sequence data indicated that the genomic region involved was a fragment of the CTV p27 gene. Amplifications of this fragment were carried out by RT-PCR and cloned in a pGEM-T vector. Eighteen of 20 Spanish CTV isolates produced an amplified fragment of the same size (approx. 350 bp), regardless of the biological properties of the isolate. Amplified p27 gene fragments of several clones were sequenced. The clones of two severe isolates showed about 90% sequence identity with the published sequence of the Florida T36 sequence. Clones derived from mild isolates showed a clear dichotomy. Three were very similar to one another and very different from those obtained for severe strains (about 40% sequence identity). However, other clones also derived from the same mild isolates were very similar to those obtained from severe isolates. These results provide evidence for the heterogeneity of CTV populations present even in "purified" isolates, and may provide a method to detect severe components contaminating mild isolates that might be used for cross protection.

### Cloning and Expression in *E. coli* of CTV Coat Protein Gene and Production of an Antiserum

M. L. P. N. Targon, O. Nikolaeva, K. L. Manjunath, R. F. Lee, and M. A. Machado

ABSTRACT. CTV dsRNA corresponding to ssRNA replicative form was isolated from 1 g of infected lyophilized bark tissue from sweet orange cv. Pera Ipigua and used as template for the first strand cDNA synthesis by MuMLV reverse transcriptase. CTV coat protein gene was amplified by PCR using specific primers. The product of amplification was cloned in the expression vector pMALc2, and *E. coli* DH-5 was transformed. The induction of expression was done with IPTG and the protein was expressed as a fusion product with a fragment of *E. coli* maltose binding pro-

tein. Fusion protein was purified by amylose resin affinity column chromatography and used as antigen to produce an antiserum. Coat protein yield was 0.25 mg/10 ml of culture. The antiserum obtained reacted with CTV coat protein with titer from 1:30,000 to 1:100,000, depending on the virus concentration in the infected tissue. The coat protein gene was sub-cloned in pUC 118, sequenced, and compared with other strains.

## **Convenient Detection of Citrus Tristeza Virus by the Simple Soaking of Stem Slices in Wells of the ELISA Plates (sELISA)**

**A. Hadjinicolis, M. Mawassi, R. Gofman, Y. Ben-Shalom, A. Kyriakou, D. Timar, and M. Bar-Joseph**

**ABSTRACT.** Incubation for 3 to 18 hr of freshly cut stem slices soaked in antibody-coated ELISA wells containing phosphate-buffered saline-Tween-20 (PBS-T), enabled the sensitive diagnosis of a wide range of citrus tristeza virus (CTV) isolates in various citrus species and varieties. Several parameters applicable for routine diagnosis of CTV using the soaking methods (sELISA), including selection of tissues, conditions of sample storage and the possibilities of composite samples are examined. A further modification of the sELISA method that included collecting and soaking peeled and unpeeled stem slices enabled us to develop a simple method for the quantification of the stem pitting reaction in sour lime plants. The sensitivity of the method was considerably improved by including antibodies from chicken immunized with a preparation of CTV coat protein expressed in *E. coli*. The practical advantages of sELISA include considerable (>50%) savings in labor, laboratory space and homogenization equipment. In addition, the method does not involve noisy operation and, thus, enables an ecologically friendly working space for CTV detection. The method is now widely applied for CTV detection in several laboratories in Israel and Cyprus.

## **Indexing of Gou Tou Cheng Seeds for Citrus Tristeza Virus**

**Chen Guoqing, Wang Hongxiang, and Yan Senxiang**

**ABSTRACT.** Seeds collected from Gou Tou Cheng seedling trees showing stem pitting symptoms were indexed for citrus tristeza virus (CTV) by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). CTV was found in the internal seed coat and in peeled seeds, including the embryo and endosperm, whereas the external seed coat of Gou Tou Cheng and seeds of trifoliolate orange were negative. The seeds of Gou Tou Cheng remained CTV positive after 1-2 yr storage at 4°C. In some cases, CTV transmission through seeds of Gou Tou Cheng was detected by DAS-ELISA.

*Key words.* Gou Tou Cheng sour orange, citrus tristeza-stem pitting virus, seed transmission

## **Status of Tristeza in Non-Commercial Citrus in Texas**

**M. Skaria**

**ABSTRACT.** Citrus tristeza virus (CTV) symptoms are not obvious in commercial citrus grown on sour orange rootstock in Texas. However, low incidence of CTV was detected by enzyme-linked immunosorbent assay (ELISA) in several commercial samples. Establishment of the brown citrus aphid in the Caribbean Islands stimulated interest in the study of possible presence of CTV inoculum in non-commercial citrus in Texas. A survey was initiated to study the incidence of CTV in citrus variety blocks and dooryard citrus trees in the Lower Rio Grande Valley, and in small citrus plantings of the Coastal Bend area near Houston. ELISA positive tests for CTV were obtained from 10 cit-

rus selections from two citrus variety blocks containing a total of 247 citrus species and cultivars. ELISA results indicated that CTV was not spread naturally in the variety blocks from 1993 to 1995. CTV incidence was 0.62% (4 in 639) among dooryard citrus in the Valley. CTV was detected in 19 of 107 samples collected from dooryard and small citrus plantings in the Coastal Bend area. Citrus in the Coastal Bend area of Texas included illegally imported satsuma mandarins and navel oranges.

## **Four Decades of Eradication and Suppression Attempts for Citrus Tristeza Virus in Israel**

**M. Bar-Joseph, Y. Oren, and D. Timar**

**ABSTRACT.** Natural spread of a severe CTV isolate was first noted in Israel in 1970 and a program was established to eradicate the virus-infected trees. Early efforts in an area of about 80,000 trees were effective and the incidence of diseased trees was reduced from 150 to 10 new cases of infection within 10 years. ELISA tests conducted during 1978 revealed hundreds of symptomless trees in a plot located near an old introduction plot. Surveys were then conducted throughout the citrus-growing area to estimate the incidence of symptomless infections. The results indicated that the disease had not spread widely and justified eradication. Between August 1979 and December 1980, the Virus Laboratory, cooperating with the Division of Citriculture and the Plant Protection Services, conducted 1.25 million ELISA tests and 0.127% of the trees surveyed were detected to be infected. Low infection rates continued during 1981 but, from 1982 onward, the percent diseased trees increased steadily and, by 1985, approximately 18,600 trees in 623 groves were infected. Statistical projections based on the observed CTV incidence, infection rates, and the probability of detection suggested that CTV had spread in a 5,000 ha area with an estimated 50,000 additional undetected infected trees. The growers and government agencies were faced with the difficult question if the eradication program should be continued. The problem was further complicated because the majority of infected trees were symptomless. To validate the statistical estimates, the survey was extended and 1.5 million additional trees were tested in 1986. The results confirmed that the disease was widespread in the coastal area and, as the result, the program was discontinued there. Recent surveys in the peripheral parts of Israel (about 30% of the total citrus area) indicated that the disease had spread to several new plots, probably by infected nursery plants. Surveys and eradication are in progress to suppress the disease in the newly planted areas.

## **Effect of Tristeza Decline in a Commercial Grove of Valencia on Sour Orange Rootstock in the Florida Flatwoods**

**John Crum**

**ABSTRACT.** A 405 ha grove of Valencia sweet orange on sour orange rootstock was planted in 1961 in the flatwoods near Indiantown, Florida. Until 1991, the tree loss rate was 0.5% or less. In 1991, declining trees with symptoms typical of quick decline were observed and presence of decline strains of citrus tristeza virus (CTV) were verified by serological tests using MCA13 monoclonal antibody. The production figures from 1979 to 1994, when the grove was replaced, are presented along with tree decline rates due to CTV. Early management strategies attempted for CTV control included replanting with CTV-tolerant rootstock and replanting with horticulturally superior looking trees on sour orange. However, the tree decline rate increased with time, reaching 30% in one year. In 1994-1995, the grove was replaced using CTV-tolerant rootstocks in a high density planting, and the opportunity was utilized to improve the drainage and irrigation systems.

## **Detection of a Mechanically Transmissible Virus in Washington Sanguine Blood Orange Trees Imported from Morocco**

**Chen Guoqing, Wang Hongxiang, and Yan Senxiang**

**ABSTRACT.** Chlorotic ringlike patterns, ringspots, ring etching and mottling, vein clearing and vein banding were observed in young and mature leaves of Washington Sanguine Blood

orange trees imported from Morocco in the 1960's. When these trees were indexed by bud-inoculation to citrus indicators and by stem slash-inoculation of Mexican lime seedlings and Etrog citron, symptoms resembling those of citrus ringspot virus (CtRSV) were observed. The graft-inoculated seedlings of Madam Vinous sweet orange, Duncan grapefruit, Etrog citron, Mexican lime, *C. excelsa*, and Gou Tou Cheng showed a shock reaction with leaf shedding in the first flush. In subsequent flushes, young leaves showed chlorotic spots and ringlike patterns, vein clearing, etching and chlorotic mottling. Oak-leaf pattern was observed in young leaves of Madam Vinous sweet orange. Young leaves of stem slash-inoculated seedlings of Mexican lime and Etrog citron showed chlorotic spots and yellowish blotches. A virus was mechanically transmitted by sap-inoculation from the originally introduced source trees and from symptomatic index plants to *Chenopodium quinoa*. Chlorotic to necrotic local lesions developed on inoculated *C. quinoa* leaves 4 to 6 days after inoculation, but no systemic symptoms were observed in new leaves.

*Index words.* Citrus ringspot virus, symptoms.

## Detection and Elimination of Citrus Tatter Leaf Virus

Wu Taishu, Du Yuying, Li Lunying, Deng Shixin, Wu Shipan,  
and Luo Zhida

**ABSTRACT.** Indexing for citrus tatter leaf virus (CTLV) on Rusk citrange showed the virus to be widespread on many cultivars including Tankan Xuegan, Gailian Cheng orange and Taiwan tankan on the Yancun Overseas Chinese Citrus Farm in Guangdong Province, China. Plants of these cultivars were placed in a glass box and subjected to thermotherapy by alternating temperatures of 40 to 50°C for 8 hr per day in for 14 to 56 days. This was followed by shoot-tip grafting. This process eliminated CTLV in 82.6% of the treated plants.

## Non-Association of Citrus Tatter Leaf Virus with Yellow Ring Disease

Wu Taishu, Deng Shixin, and Wu Shipan

**ABSTRACT.** Trees of Anlin and Gailian Cheng orange on trifoliate orange rootstock developed a yellow ring pattern under the bark at the budunion. Although the budwood had come from citrus tatter leaf virus-(CTLV) free sources, all the affected trees were indexed for CTLV to determine whether they had become infected with the virus and whether it was associated with the symptom. All the trees were found to be CTLV-free. The yellow ring is, therefore, not associated with CTLV and may be caused by an incompatibility between these cultivars and trifoliate orange.

## Detection and Characterization of Viruses Associated with a Dwarfing Disease of Satsuma Mandarins in New Zealand

M. Aftab, M. N. Pearson, and P. Mooney

**ABSTRACT.** A destructive disease on satsuma mandarin cultivars Ishikawa, Matsuyama, Miho, Miyagawa and Okitsu has been observed showing dwarfing plus leathery, reduced and boat-shaped leaves. Infected plants produce fewer and smaller fruit. Several samples were tested against a polyclonal antibody to satsuma dwarf virus and gave negative results. Samples from diseased plants were mechanically inoculated to 20 indicator hosts, but only *Phaseolus vulgaris* cvs. Top Crop and Red Kidney were susceptible, exhibiting systemic chlorotic lesions. Purification from leaf and bark tissue of dwarfed trees revealed mixed infections of three morphologically distinct particles; rigid rods, flexuous rods and spherical particles. The rods appeared to be fragmented and variable in length, both the rigid and flexuous rods ranging from 25 to 350 nm (modal

length 51-100 nm). The spherical particles are approximately 24 nm in diameter. In tests with Dweet tangor and Thorney mandarin seedlings, Dweet tangor exhibited leaf flecking in field trees and both cultivars contained rod-shaped and spherical particles. Polyclonal antibodies to the particles have been produced and studies are continuing.

## **Satsuma Green Wilt: A New Citrus Disease**

**Shu Guang Ping and Wu De Xi**

**ABSTRACT.** The Hunan Province of China has about 200,000 ha planted with satsuma. Since 1975, a new citrus disease known as satsuma green wilt has occurred in 10 counties. The incidence of this new disease ranges from 2 to 23% and it causes serious damage including a rate of dead trees of 0.7 to 11% in some orchards. The leaves of affected trees appear suddenly wilted and rolled, but they keep their green color and do not fall immediately. Wilting begins at the top of the tree and moves down through the canopy. Twigs dry up within 10 days and sometimes the whole tree dies. Diseased trees show budunion crease with brown tylosis-like structures and zinc accumulation in the xylem of the scion near the budunion. The disease has appeared periodically (at approximately 10-yr intervals) and symptoms are mostly observed in April-May and in the harvesting season. The cause of this new disease is presently unknown, but heavy pruning and proper management of water and fertilizer when the leaves begin to wilt and roll seem to improve some affected trees and are recommended practices.

## **Symptoms and Characterization of the Isoenzymes Induced by Several Citrus Viroid Isolates**

**Wu De Xi and Hu Qin Xue**

**ABSTRACT.** Seven citrus viroid isolates including CEV were collected from different districts and citrus cultivars in Hunan Province. Five were from local cultivars and two were isolated from Miyamoto satsuma and Newhall orange, introduced from Japan and the U.S., respectively. Field trees were grafted on trifoliolate orange and showed a variety of symptoms including stunting, low vigor, and reduced leaf size, but only two of them showed bark scaling in the rootstock. Upon inoculation on Etrog citron 861-S-1, most isolates induced necrosis at the leaf tip, midvein and petiole, leaf epinasty; and two of them also induced stunting and leaf kinking. Leaf extracts from the inoculated citron plants were used to characterize the isoenzyme patterns of esterase, peroxidase, and polyphenol oxidase induced by the different isolates. All the inoculated plants showed a reduced number of polyphenol oxidase bands, compared with uninoculated control plants; no obvious difference was detected in the esterase isoenzyme bands among isolates; and differences in the number and location of the peroxidase bands were observed in some isolates.

## **Temperature Effects on Replication of Citrus Viroids in Callus Culture**

**M. T. Gleeson, M. R. Gillings, P. Broadbent, C. Dephoff, N. Franks, and I. Barchia**

**ABSTRACT.** Internodal tissue of Etrog citron (Arizona 861S) infected with one or more known citrus viroids was used to establish callus cultures on an amended Murashige and Skoog medium. Cultures were raised in the dark at a constant temperature (25°C) for 6 wk. Callus from each culture was then divided and grown on fresh medium in the dark at five constant temperature treatments (15, 20, 25, 30 and 35°C) and a diurnal treatment (32/20°C) for a further 6-wk-period. Increase in tissue weight was recorded for each sample. Total nucleic acids were extracted from each sample and the concentrations of DNA and RNA per g of callus were measured spectrophotometrically.

metrically. Equivalent amounts of total nucleic acids were analyzed by sequential-PAGE. The relative intensities of viroid bands were determined by densitometry scans. Within cultures containing the same viroid, marked variation in band intensity was found over the temperature range tested. The temperature at which intensity was greatest varied among the different viroids tested, from 30°C for CEVd to 20°C for CVd-IIb. The optimum temperature for replication for most viroids tested was 25°C. When more than one viroid was present in the plant, an interference effect was noted. The optimum temperature for CEVd in a mixed infection rose to 35°C. The applicability of these results to biological indexing is discussed.

## **Incidence and Control of Citrus Exocortis of Several Counties in Sichuan Province of China**

**Li Longhua, Huang Zhiyuan, Zhang Jing, Qi Juxian, and Liang Anguo**

**ABSTRACT.** Citrus exocortis viroid (CEVd) causes a serious disease on citrus grafted onto *Poncirus trifoliata* and other susceptible rootstocks worldwide. A survey based on symptom observation was carried out in 11 counties of the Sichuan Province in China. Fourteen of the fifteen cultivars inspected were found CEVd-infected with variable incidence. Valencia, Cadenera and Robert navel sweet orange, and Eureka lemon, were the most affected cultivars, with disease incidence ranging from 32.2% to 100%, followed by Jingchen sweet orange with a CEVd incidence between 5 and 22.2%. Satsuma and tangerines were barely affected. Diseased trees were dwarfed and fruit yield was reduced by 50 to 70% compared with symptomless trees. Recommended control measures include development of virus-free plants of selected cultivars by shoot-tip grafting and indexing on indicator plants, establishing virus-free mother blocks, propagating only virus-free budlings to establish commercial orchards, and disinfecting tools before cutting on CEVd-free plants.

## ***Spiroplasma citri* Detection by Immuno-Capture PCR**

**Colette Saillard, A. Nhami, P. Moreno, Monique Garnier, and J. M. Bové**

**ABSTRACT.** In *Spiroplasma citri*-specific immuno-capture PCR (IC-PCR), the spiroplasmas from crude extracts are captured by *S. citri*-specific polyclonal antibodies coated on the walls of the PCR tubes. The tubes are emptied and washed and then PCR is performed as usual. The PCR primers are derived from the sequence of *S. citri* virus SpV1 strain R8A2B. This technique was evaluated on *S. citri*-infected greenhouse trees in Bordeaux and has now been applied to trees from various regions in Spain and Morocco. Our results were not as satisfactory as expected, since some trees with typical symptoms of stubborn gave negative IC-PCR results. Rather than improving the IC-PCR procedure for specific pathogen detection, we are now developing a PCR method which involves the partial purification of leaf DNA, irrespective of the phloem- or xylem-restricted pathogens to be detected (*S. citri*, *Phytoplasma aurantifolia*, *Liberobacters* or *Xylella fastidiosa*). The detection of any one of these pathogens will be based on the use of pathogen-specific primers.

## **The Northward Movement of Citrus Psylla (*Diaphorina citri* Kuwayama) in Guangxi, China**

**Qiu Zhu-shi, Zhou Qi-ming, and Quan Jin-cheng**

**ABSTRACT.** The citrus psylla (*Diaphorina citri*) has long been present in the southern and central parts of Guangxi, but prior to 1980, it was not found in most of the northern counties.

Since then, however, two northward migrations of the psyllid in 1982 and in 1987 established it in all seven counties in the north. This movement has been attributed to southerly winds coinciding with the peak psylla population months of June to August; the warmer winters of 1982 and 1987 when the mean monthly temperatures of December and January were 2°C above normal; and with the increased planting of citrus in the north where previously there were only isolated orchards.

## **Seasonal Abundance and Natural Enemies of *Diaphorina citri* (Hemiptera: Psyllidae) in Citrus Orchards of São Paulo State, Brazil**

**S. Gravena, M. J. G. Beretta, P. E. B. Paiva, R. Gallão,  
and P. T. Yamamoto**

**ABSTRACT.** Surveys for *Diaphorina citri* (Homoptera: Psyllidae), the vector of huanglongbing (HLB) (greening), were conducted in São Paulo State, Brazil, to determine its seasonal abundance and to identify its natural enemies. This study was done to obtain information which would be needed to control HLB should it ever occur in Brazil. The highest psyllid densities were observed from December to March, but psylla could be found year round. No parasitoids were observed, but the coccinellid, *Scymnus* sp., was associated with the psyllid.

## **Temporal and Spatial Dynamics of Citrus Blight in Brazil**

**F. F. Laranjeira, N. Guirado, M. J. G. Beretta, K. S. Derrick,  
and R. F. Lee**

**ABSTRACT.** The temporal and spatial progress of citrus blight in Brazil was evaluated in three areas of São Paulo state between 1986 and 1992 and in one area of Minas Gerais state between 1983 and 1986. The trees were recorded as blighted or non-blighted by means of visual assessment and the water uptake test. The last evaluation in Minas Gerais was monitored using the 12 kDa protein test. The first two areas in São Paulo yielded non-useful data due to inconsistent evaluations. The data of the two remaining areas were analyzed using five disease progress models (Exponential, Linear, Gompertz, Monomolecular and Logistic) and the spatial data was studied by means of Two-Dimensional Distance Class Analysis, dispersion index, Geostatistics, runs and centroid analysis. The temporal progress in the remaining São Paulo area showed a linear trend which is typical of non-infectious diseases, whereas the Minas Gerais data adjusted better to the exponential model. These extremely different responses could be assigned to different cultural practices; in the first area, affected trees were periodically removed and new trees planted; in the second area, tree removal did not occur. The spatial patterns did not differ significantly between the two areas. No significant discrete cluster was observed in the areas or near the edges. No significant focus could be localized and the centroid did not appear to move in any direction. Initially, affected trees appeared at random but, with time, some progressive clustering occurred. Blighted trees tended to be more clustered within rows than across rows.

## **The Damage Caused by Virus and Virus-Like Diseases of Citrus and Measures for Control in Anhua County, Hunan Province, China**

**Liu Gan-sheng and Chen Guo-jun**

**ABSTRACT.** Citrus production in Anhua County in Hunan Province, China, only began in the mid-1970s. Unfortunately, the lack of knowledge of virus and virus-like diseases in the county at

the time and the failure to adopt strict preventative measures resulted in the rapid spread of diseases and consequent economic losses. A program to produce virus-free plants has now been adopted to prevent future losses.

## **Indexing for Viruses and Viroids and the Production of Disease-Free Plants by Shoot-Tip Grafting in São Paulo, Brazil**

**M. A. Machado, S. A. Carvalho, C. R. Baptista, J. L. Silvério,  
and G. W. Müller**

**ABSTRACT.** The use of citrus tristeza virus (CTV)-free rootstocks and nucellar clones saved the citrus industry in São Paulo, Brazil. Many old line parent trees, from which the nucellar lines were obtained, are still kept in the germplasm collection of the Centro de Citricultura Sylvio Moreira in Limeira. They are no longer used since they are infected with several viruses and viroids. In addition, the nucellar lines had been naturally infected with CTV and mechanically transmissible viroids. Since 1991, the Centro de Citricultura has been conducting a program to index all varieties and clones, and to subject them to shoot-tip grafting (STG). The varieties include 62 nucellar and 151 old line clones consisting of 123 sweet oranges, 46 mandarins and hybrids, 19 lemons, 6 grapefruits, 3 tangelos, 5 limes, 4 citrons and 7 others. Indexing on Mexican lime showed that 26% of the CTV isolates were severe, and were mostly in sweet orange, grapefruit and citron. All those from sweet orange induced seedling yellows in sour orange indicator plants. Nucellar scions were all exocortis-free, but 37% of the old lines were infected. Some psorosis was found in old-line varieties. STG has eliminated all the CTV and exocortis but, in a few instances, psorosis was still present. Cachexia indexing is still underway. Disease-free plants are being aphid-inoculated with mild CTV isolates for cross protection.

## **Improvements to the Shoot-Tip Grafting Method for Citrus**

**Jiang Ling and Wan Shu-yan**

**ABSTRACT.** Three modifications to the shoot-tip grafting method for citrus were studied. The traditional inverted T-cut in the rootstock seedling was compared with a smaller wedge cut where the removed tissue is used to press the tip into place. The graft transmission rate increased from 38.5 to 58% to 60 to 80% using nine varieties, and it was found that fewer adventitious shoots developed after grafting with the new method. In addition, soaking the shoots before grafting for 10 min in 0.5 ppm 6-benzylamino purine led to a higher survival rate, as did the use of autoclaved sand and vermiculite for the transplanted plantlets and then sprayed with Hoaglands solution containing 0.1% urea and 0.1%  $\text{KH}_2\text{PO}_4$ .