Literature Cited

Henny, R. J., A. R. Chase and L. S. Osborne. 1992. Aglaonema production guide. Institute of Food and Agricultural Sciences, Central Florida Research and Education Center. Apopka Foliage Plant Research Note RH-91-2.

Poole, R. T. and A. J. Pate. 1980. Bent-Tip of *Aglaonema commutatum* 'Silver Queen'. Foliage Digest. September:15-16.

McConnell, D. B., R. W. Henley and K. B. Kelly. 1989. Commercial foliage plants: twenty years of change. Proc. Fla. State Hort. Soc. 102:297-303.

Proc. Fla. State Hort. Soc. 110:110-112. 1997.

DIRECT TISSUE BLOT ADAPTED FOR USE IN DETECTING CMV IN GLADIOLUS

M. S. ELLIOTT AND F. W. ZETTLER
Department of Plant Pathology
University of Florida
Gainesville, FL 32611

Additional index words. Cucumber mosaic virus, anti-rabbit alkaline phosphatase conjugate, cross-absorption, NBT, BCIP.

[redacted]

This study was undertaken to provide a rapid, simple and reliable test for screening plants and bulbs for CMV. The direct tissue blotting procedure was adapted so that it could be utilized by non-scientifically trained staff in existing facilities on site.

Materials and Methods

Symptomatic gladiolus leaves, corms and roots were selected to determine which tissue is best suited for direct tissue

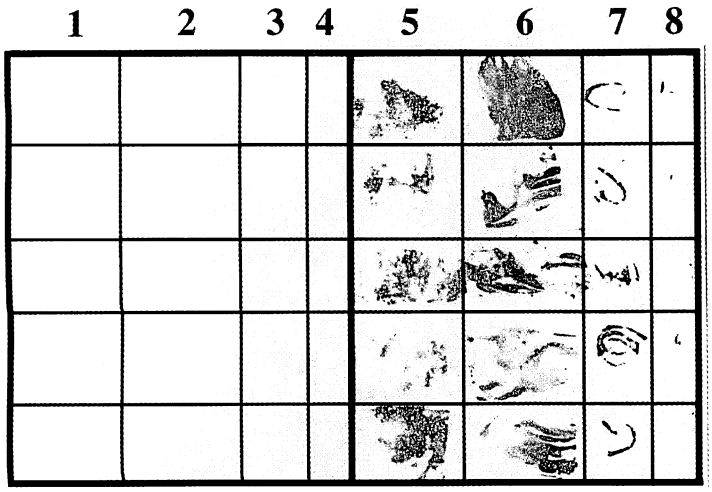

Figure 1. Nitrocellulose membrane blotted with healthy gladiolus tissue, columns 1-4 and CMV-infected gladiolus tissue, columns 5-8. Left to right, column 1,5 = old corm, 2,6 = new corm, 3,7 = leaf, 4,8 = root.

were eliminated by incubating the membrane in 20% bleach (1.0% sodium hypochlorite) for 2-3 minutes and rinsing in distilled water.

Results and Discussion

Cross-absorption of CMV antiserum in healthy plant sap and blocking solution eliminated nonspecific, non-virus reactions which can lead to erroneous results. Positive reactions were observed in blots of all CMV-infected tissues tested (Fig. 1).

A minimum number of inexpensive materials and reagents, all of which are readily available from scientific supply companies are required (Table 1). The procedure has been modified making it easily accessible to non-scientifically trained personnel, and it may be completed in less than 1 day (Table 2). Reagent volumes and equipment may be adjusted to meet the indexing requirements and many of the reagents may be prepared in advance and refrigerated or frozen. For example, extracted healthy sap and blocking solution may be frozen and stored indefinitely. The mixture of blocking solution, healthy sap and antiserum may be frozen and re-used 2-3 times. Stock solutions of anti-rabbit alkaline phosphatase conjugate may be prepared and refrigerated at 2-4C for as long as 3 months. Likewise, membranes may be blotted and stored for several months in a dry location prior to testing. The antiserum/blocking solution may be prepared and refrigerated 1 day prior to use, but the substrate developing solution must be prepared immediately prior to use.

While reactions were observed in all infected tissues tested, corm tissue harvested at the end of the season proved to be most satisfactory. Root tissue was frequently absent or degenerated and unsatisfactory for blotting. Leaf tissue was satisfactory, however, this tissue would be absent when corms are purchased from outside sources or following storage. The virus appears to be evenly distributed throughout the corm tissue, and it is readily detectable at all stages of production and storage.

Sampling strategies: Gladiolus bulbs which had become infected with CMV the previous season generally develop "white break" symptoms upon sprouting. These are often rogued and destroyed. Gladiolus plants which become infected after they mature and after the flowers have been harvested, will not develop symptoms until they are planted the following season. Thus, the grower does not know the health status of the crop prior to digging and processing for the next season. Inasmuch as digging, processing, storage and re-planting costs are substantial, knowing the health status of the bulbs

Table 1. Buffer Solutions and Materials.

Material			Source	
TBST Buffer				
	0.02 M Tris	3.15 g	Fisher:	153-500
	0.15 M NaCl	8.7 g	Fisher:	S271-3
	1.0% Tween 20	1.0 ml	Fisher:	BP337-100
	Adjust to pH 7.2 in 1.0 liter distilled water. Pre-mix and refrigerate.			
Substrate Buffer				
	0.1 M NaCl	5.84 g	Fisher:	S271-3
	0.1 M Tris	15.76 g	Fisher:	153-500
	Adjust to pH 9.5 in 1.0 liter distilled water. Pre-mix and refrigerate.			
Substrate				
	0.1 M $MgCl_2$	20 ul	Fisher:	M33-500
	Pre-mix 1.0 g $MgCl_2 \cdot 6H_2O$ in 50 ml de-ionized H_2O; store.			
	0.1 M NBT (nitro blue tetrazolium)	20 ml	GIBCO/BRL:	1820-016
	0.1 M BCIP (bromo-chloro-indolyl phosphate)	20 ul	GIBCO/BRL:	1820-016
	substrate buffer	20 ml		
	Mix just prior to use.			
Cucumber Mosaic Virus Antiserum			*ATCC: ATCC PV-30	
anti-rabbit alkaline phosphatase conjugate			Sigma: A3687	
nitrocellulose membrane			Bio-Rad: 162-0115	
petri dishes			Fisher: 8-757-12	
pipette tips 200 ul			Fisher: 21-278-51	
pipetter 2-20 ul			Rainin Pipetman	
latex gloves			Fisher	
shaker			Fisher	
magnetic stirrer & stir bars			Fisher	
bleach				
powdered skim milk				

*ATCC = American Type Culture Collection

Table 2. Direct tissue blotting procedure.

1. Prepare buffers (see Table 1).
2. Blot tissue on nitrocellulose membrane.
3. Extract sap from healthy tissue in TBST (1 g tissue:5 m TBST).
4. Incubate the membrane with shaking for 1-2 hours at room temperature in the cross-absorbed CMV antiserum prepared by diluting antiserum 1:1000 in a solution consisting of equal volumes of blocking solution and the healthy sap extracted in TBST (step 3).
5. Wash in TBST, with shaking, 5 min. Repeat 2 more times.
6. Dilute the anti-rabbit alkaline phosphatase conjugate 1:30,000 in blocking solution. Ex. 1:30,000 = 0.67 µl conjugate in 20 ml of blocking soln. This solution can be frozen and re-used about 3 times.
7. Incubate the membrane in the anti-rabbit alkaline phosphatase conjugate, on a shaker for 1 hour at room temperature.
8. Wash in TBST with shaking 5 min. Repeat.
9. Wash in substrate buffer with shaking 5 min.
10. Mix the substrate. Begin mixing the substrate during the last wash. Do not make it sooner as it is light sensitive.
11. Incubate membrane in substrate in the dark until reaction is adequate. Do not shake.
12. Stop the reaction with distilled H_2O.
13. Clear any chlorophyll or bulb tissue stains by incubating a few minutes in 20% chlorox (to give a final solution of 1% sodium hypochlorite).
14. Rinse off the chlorox with distilled H_2O.
15. Allow the membrane to dry on paper towels and store in a ziplock bag, protected from light.

can have a significant economic impact. By testing a statistically significant number of bulbs, this blotting technique could be used to determine if the percentage of bulbs that remain virus-free after growing in the current season is great enough to warrant harvesting as opposed to being destroyed.

Direct tissue blotting can also be used to determine the virus status of bulbs prior to purchasing from outside sources. Likewise, this method can be used to index bulbs prior to placing meristems in culture for rapid multiplication.

Literature Cited

Elliott, M. S. and F. W. Zettler. 1996. Bamboo mosaic virus detected in ornamental bamboo species in Florida. Proc. Fla. State Hort. Soc. 109:24-25.

Lin, N.-S., Y.-J. Chai, T.-Y. Huang, T.-Y. Chang and Y.-H. Hsu. 1993. Incidence of bamboo mosaic potexvirus in Taiwan. Plant Dis. 77:448-450.

Yang, T.-C., F. W. Zettler and J. E. Polston. 1996. Direct tissue blotting (DBT) used to detect lily × potexvirus (LVX). Phytopathology (Abstr.) 86:S72.

Proc. Fla. State Hort. Soc. 110:112-114. 1997.

FLORIDA GREENHOUSE GROWER SURVEY TO PRIORITIZE RESEARCH AND EXTENSION EFFORTS

KIMBERLY A. KLOCK AND MARIA BRAVO
University of Florida, IFAS
Fort Lauderdale Research and Education Center
3205 College Avenue
Fort Lauderdale, FL 33314

DAVID G. CLARK
Department of Environmental Horticulture
University of Florida
Gainesville, FL 32611

Additional index words. Ornamental growers, greenhouse industry, foliage industry.

Abstract. **A survey of Florida greenhouse growers was conducted to assess research and extension priorities in floriculture. Florida growers were split into three groups based on their gross sales for 1995. Regardless of gross sales, all growers rated growing a quality crop, markets, financial concerns, and pest management as important issues. The majority of growers felt that they were able to acquire adequate information pertaining to greenhouse issues. Small growers (based on 1995 sales) reported getting their information from in-state extension agents, in-state extension publications, and trade journals while medium-sized growers reported getting their information from trade journals, professional association newsletters, and sales representatives. Large growers' main source of information was from professional association newsletters and sales representatives. Fifty-seven percent of the growers surveyed reported that they had contributed financial support for research in problem areas they encountered in their business. The results of this survey suggest that floriculture research in Florida should concentrate on crop production issues and that growers are getting the information they need from a variety of sources.**

Florida Agricultural Experiment Station Journal series no. N-01448. Mention of any trade names does not constitute an endorsement. We wish to thank our colleagues who helped develop this survey as well as the many growers who took time to answer the questions.

The United States produces approximately $3.4 billion worth of floriculture products annually, ranking it third in the world (Hamrick, 1996). Florida produced more than $660 million of floriculture products in 1995, ranking it second in the United States (USDA, 1997). Florida growers produce potted plants, hanging flowering baskets, cut flowers, bedding and garden plants, ferns, and foliage plants.

Because of the wide variety of floriculture crops grown in Florida as well as the large number of growers in the state, it is difficult to assess research and extension priorities. Floriculture researchers at the University of Florida in conjunction with researchers from Auburn University, Berry College, Louisiana State University, Mississippi State University, North Carolina State University, and the University of Tennessee wrote an 11 question survey on greenhouse operation and management issues to learn growers opinions about problem areas they encounter in their business. It was the intent of this survey to help researchers prioritize research and extension efforts based on issues of concern to growers. Information from Florida growers was extracted and is presented here.

Materials and Methods

The 11-question survey consisted of two types of questions. The first type of question asked growers to rate various issues on a scale of 1 to 6 with 6 being issues of great importance and 1 being issues of no importance. The other type of question asked growers to circle all responses that applied to their business.

The survey was distributed to ≈400 growers in the state of Florida in spring 1996 with a return rate of ≈20% (21% of responses from northern Florida, 55% of responses from central Florida, and 23% of responses from southern Florida). Based on gross sales for 1995, the growers were divided into three groups: group 1 with sales of 0 to $99,999 (14%), group 2 with sales of $100,000 to $999,999 (55%), and group 3 with sales > $1,000,000 (31%). The majority of group 1 growers had ≈10,000 to 25,000 sq.ft. of greenhouse production under cover and/or ≈0 to 0.25 acres of production space not under cover. The majority of group 2 growers had 10,000 to 50,000