

INCIDENCE AND EXPRESSION OF CYMBIDIUM MOSAIC VIRUS
IN DENDROBIUM HYBRIDS

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By

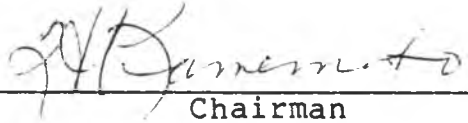
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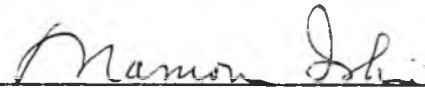
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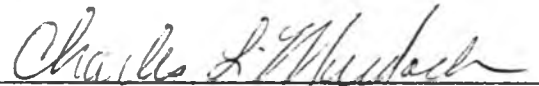
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ABSTRACT

The incidence of cymbidium mosaic (CyMV) virus and its effect on yield and necrotic symptom expression in flowers were investigated in Dendrobium cultivars and selections. The percentage of plants infected with CyMV was found to increase linearly with time spent in cultivation. Infection increased approximately 15% per year for plants 4 to 7 years of age.

Differences in flower spray yield between CyMV-infected and non-infected plants were not significant at the 5% level. However, in three groups of plants, the reduction in yield was significant at the 15% level suggesting that flower yields might be depressed in CyMV-infected plants.

Necrotic symptoms in flowers were found to be highly correlated with CyMV infection in 3 commercial dendrobium cut-flower cultivars. A small number of resistant plants were found in all three cultivars. Dendrobium Jaquelyn Thomas 'Uniwai Supreme' was found to have the highest percentage of resistant plants. A large amount of variability in the expression of flower necrosis was found among various Dendrobium cultivars and selections. Expression ranged from 0 to 100% necrotic flowers at various stages of raceme maturity. Expression patterns were consistent within plants and among related plants. This suggests that genetic variability for resistance to CyMV-induced flower necrosis exists in Dendrobium.

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I. INTRODUCTION

The Orchidaceae is a large and diverse group of flowering plants. It is represented by about 725 genera and 20,000 to 25,000 species (Dressler, 1981) plus countless hybrids (Royal Horticultural Society, 1976, 1981). Dendrobium is one of the largest genera in the family Orchidaceae. In Hawaii, Dendrobium hybrids are important for cut flower production because of their attractiveness, high yields and long vase life.

Cymbidium Mosaic Virus (CyMV), a widespread orchid virus found in collections throughout the world (Sheehan et al., 1981), has become a major concern of both commercial orchid growers and hobbyists (Dunn, 1980). Symptoms of CyMV in Dendrodium include foliar pitting or streaking (Dunn, 1980) and/or floral necrosis (Yuen et al., 1979). The virus may also stunt infected plants, reducing both growth and flower yields (Sheehan et al., 1981).

Symptom expression of CyMV is highly variable and is often confusing to growers and pathologists alike. The genetic makeup of the plant in conjunction with variation in environmental conditions such as light and temperature will affect symptom expression. This variability makes the use of visual symptoms a highly unreliable method of CyMV detection, although it is perhaps the method most widely used by growers (Lawson and Ali, 1975). More reliable methods

are available to detect and diagnose CyMV in orchids (Lawson and Ali, 1975).

CyMV appears to be an economically significant pathogen in Hawaii's dendrobium industry. Twenty five percent of samples submitted to the Plant Disease Clinic of the University of Hawaii, Manoa through 1973 were found to be virus infected (Ishii and Martinez, 1973), while 44% of samples submitted through 1977 were infected. Of these infected plants, 96% were determined to be CyMV or CyMV in combination with *Odontoglossum* Ringspot Virus (ORSV). A 1978 survey of the UH Manoa collection showed 62% of the plants to be virus infected. All plants over 10 years old were completely infected (Dunn, 1980).

The purpose of this study is to determine the relationship of the age of plants propagated from seed and the degree of CyMV infection of plants grown in the greenhouse and saranhouse, to examine the effects of CyMV infection on yield, and to determine if there are any genetic differences in CyMV symptom expression among some Dendrobium cultivars and selections.

REVIEW OF LITERATURE

Viruses differ from other disease inducing organisms such as bacteria or fungi in several ways. They are extremely small, not visible in the plant even with the aid of the light microscope and able to pass through porcelain filters with pores small enough to capture bacteria (Bos, 1978; Francki, 1968; Magee, 1943). They consist merely of nucleic acid with a protein coat and have been observed in various shapes and sizes (Francki, 1968). Viruses have the ability to multiply and mutate, but only inside a living host cell. Unlike bacteria or fungi, they cannot be grown on nutrient cultures (Wilson and Brown, 1953). They are systemic and persistent with the ability to multiply and spread through all parts of the host (Ishii, 1968; Jensen, 1953; Morel, 1960). Virus transmission must be aided by some external vector such as insects or man, for they are not able to independently initiate infection (Ishii, 1968). The association between the host plant and the virus organism is much more intimate than that of other disease organisms. Typically, the virus particle (viron) enters the hosts cell and sheds its protein coat. The freed viral nucleic acid acts as genetic material and takes over cell function leading to physiological and biochemical disturbances. This initiates a change in the cell protoplasm and directs the production of more virus particles (Wellman, 1972).

Jensen (1959) listed 32 virus diseases found in orchids. However, since disease names were often based on symptoms described on various plants, it is likely that some of these diseases may be caused by the same virus (Francki, 1968). The host range of orchid viruses is generally restricted to the Orchidaceae (Murakishi and Ishii, 1955; Perez and Cortez-Monllor, 1959).

Cymbidium Mosaic Virus (CyMV) is the most common and widespread orchid virus. This disease infects a wide range of orchids, induces a wide variety of symptoms, is extremely stable, exists in high concentrations, and is mechanically transmitted with ease. It is prevalent throughout the world (Ishii and Martinez, 1973).

Electron micrographs indicate CyMV to be elongate, sinuous rod-shaped particles with a typical size of 475 x 18 μ (Gold and Jensen, 1951; Kado and Jensen, 1964; Wellman, 1972). CyMV has been found to be serologically related to hydrangea ring spot virus, cactus virus X, potato virus X and white clover mosaic virus (Martyn, 1968). In orchid genera, it is the causal agent of cattleya leaf necrosis, cymbidium black streak, dendrobium mosaic, spathoglottis diamond spot and possibly other viral diseases (Martyn, 1968).

The first published report of CyMV was by Magee (1943) who described "Black disease" in a cymbidium collection in

New South Wales. Although he did not demonstrate the viral nature of the disease, he correctly inferred that a virus was the causal agent and proposed the name "orchid or cymbidium mosaic virus" (Jensen, 1953). Since then, the viral nature of CyMV has been repeatedly demonstrated.

Recent Florida surveys indicate that CyMV is found in all 17 major genera of cultivated orchids (Bodnaruk et al., 1979; Sheehan et al., 1981; Wisler et al., 1982 and 1979; Zettler, 1978). Infected orchids were found in all cultivated collections studied. Virus incidence was found to be higher in orchids that had been in cultivation for more than four years and in clonally propagated cultivars. Seedlings and plants collected from their native habitats were not infected. A survey of 13 nurseries in Puerto Rico produced similar results (Perez and Cortez-Monllor, 1959).

In Hawaii, CyMV is a serious orchid disease (Ishii, 1968). A survey of the UH Manoa collection showed 62% of plants to be infected with CyMV (Dunn, 1980).

The damage caused by CyMV infection and the symptoms produced are dependent on the interaction between the virus and the genetic makeup of the host, the environment and cultural conditions. Thus, there is a wide range in the severity of the disease produced by CyMV according to the variety of plant infected and the condition in which it is grown (Francki, 1968; Ishii, 1968).

Although symptoms may vary according to the genus affected, CyMV typically produces chlorotic and/or necrotic spotting or streaking of the leaves. The most obvious damage is to the flowers, with floral necrosis appearing several days after the flowers open (Francki, 1968; Sheehan et al., 1981). Rings, irregular lesions and leaf surface collapse have also been observed. Severely affected leaves may be killed. Conspicuous symptoms are observed 15 to 26 days after infection (Kado and Jensen, 1964). Plants infected with CyMV display a reduction in both the number and size of flowers produced (Corbett, 1960; Jensen and Gold, 1955).

Often plants go through an acute phase followed by a chronic phase of the disease. The acute phase occurs soon after infection with the virus and is characterized by severe symptoms or even death. Following this initial shock period, a chronic phase often occurs in which viral symptoms are less severe (Bos, 1978). Plants may lose viral symptoms but will continue to carry the virus. In this condition, they are still able to infect other plants and under the right environmental conditions, may again develop strong symptoms (Jensen, 1953). Resistant plants may not show viral symptoms at all. Therefore, absence of symptoms is no assurance that the plant is virus-free. Similarly, viral symptoms may not necessarily be conclusive evidence of viral infection. Plant abnormalities resembling viral symptoms

may be caused by sunburn, insect damage, fungal infection, nutritional imbalance, spray injury or other environmentally induced causes (Francki, 1968; Ishii, 1968; Kado, 1964).

It has been demonstrated that CyMV is not transmitted through seed propagation (Yuen et al., 1979). However, Sheehan et al. (1981) cautioned that when using green-pod culture, infection from placental tissue may result.

No insect vectors have been found to transmit CyMV. A study by Namba and Ishii (1971) demonstrated that two species of aphids, Myzus persicae (Sulzer) and Cerataphis orchidearum (Westwood) failed to transmit CyMV or ORSV in cattleyas, although they readily probed into test plantlets. Murakishi (1955) noted that plants placed in an open lath house for three years received very little, if any, insect transmission of CyMV.

Occurrences of CyMV in wild orchids is extremely rare, probably due to the fact that there are no known natural vectors, the virus is not seed transmitted and plants do not grow in close proximity as cultivated plants do (Bodnaruck et al., 1979). CyMV is very stable and can remain infectious for 10 minutes at 65° C or 7 days at room temperature (Jensen, 1953). Mechanical transmission is easily accomplished during propagation or harvest by knives, shears or direct contact and is probably the most common means of inoculation (Batchelor, 1982; Morel, 1960).

The absence of CyMV in wild orchids and its prevalence in cultivated collections suggest that its spread is largely due to the cultural practices of growers (Sheehan et al., 1981).

Since CyMV is difficult to diagnose and no certification program exists, CyMV and other virus disease have been widely distributed through the international exchange of plants (Sheehan et al., 1981). The outstanding hybrid Cymbidium Alexandri var. Westonbirt FCC/RHS which has proven to be exceptionally valuable in hybridization, exhibits strong virus symptoms. All divisions of this plant exhibit similar symptoms and these have come to be associated with the cultivar. This plant was widely distributed to breeders worldwide and demonstrated how quickly CyMV can invade the best of collections (Jensen, 1951; Morel, 1960).

Orchid viruses are generally restricted to Orchidaceae, so the problem of outside hosts or alternate sources is not significant. However, enormous reservoirs of CyMV exist within orchid collections and since little prevention is practiced, the problem continues to be serious. Commercially mass propagated clonal orchids, such as Vanda Miss Joaquim, are uniformly infected (Ishii and Martinez, 1973). Bornaruk et. al. (1979) feel that under conventional cultural conditions and practices, virus infection may be unavoidable.

Since CyMV is generally spread by cultural and propagation methods, orchid growers must vigorously practice sanitation methods. Ishii (1968) recommends a certification program as done in the ornamental industry.

In order to identify CyMV-infected plants, it is necessary to detect the presence of the virus. At present, four methods are available to do this.

- 1) Visual Symptoms - Visual symptoms of CyMV infection have repeatedly been shown to be highly variable and thus unreliable. The same virus may be symptomless in some hosts and cause severe damage in others. Environmental conditions may also affect symptom expression. No published guides are available to aid diagnosis. This method is not recommended for CyMV diagnosis (Bertsch, 1982; Lawson and Ali, 1975).
- 2) Bioassay - In this method, CyMV is transmitted from the orchid to a suitable test plant by wound inoculation. Cassia occidentalis (L.) has been shown to be a reliable and easily grown indicator plant for CyMV testing. Its advantages is that it is highly reliable, low cost, test plants are readily available, and applicable to small scale operations. Its disadvantage is that it is time and space consuming (Corbett, 1960; Hollings, 1974, 1966; Lawson and Ali, 1975).

- 3) Serology - In this method a immunological reaction between the antigen (viral protein) and its specific antiserum triggers a visible reaction indicating the viruses presence. The plant virus research group at the University of Florida (T. J. Sheehan, G. C. Wisler and F. W. Zettler) aided by American Orchid Society funds has worked to simplify and reduce costs of the immunodiffusion technique. The advantages of this technique is that it is specific, rapid, reliable and economically feasible. The disadvantage is the availability and/or production of antiserum (Bertsch, 1982; Bodnaruck et al., 1978; Lawson and Ali, 1975; Sheehan et al., 1981; Wisler et al., 1979).
- 4) Electron Microscopy - This method offers the only direct method of confirming the presence of CyMV. the other methods being based on reactions to the virus. It is useful in clinical detection, diagnosis and identification. Its disadvantage is that it requires specialized equipment and training, and a large capital investment and operating expense (Bertsch, 1982; Lawson and Ali, 1975).

At present, no direct control of CyMV exists. Unfortunately, plants do not develop protective antibodies to viral infections as do animals. Therefore the development

of a vaccine or serum is not a possibility. At best, the spread can be controlled by the isolation or destruction of infected plants and by observing strict sanitation practices (Wilson and Brown, 1953).

Attempts have been made to obtain virus-free plants through meristem tissue culture. Morel (1960), the father of orchid tissue culture, had some success with cymbidiums. However, the distribution of the virus in apical and lateral buds is poorly understood and attempts to free cattleyas from CyMV have been largely unsuccessful. This is thought to be due to the large size of tissue (shoot tip) required to culture cattleyas (Ishii, 1974; Lawson and Hearon, 1974). Unfortunately, the tissue-culture method to free plants of CyMV has proven unreliable and commercial labs have abandoned it (Bertsch, 1982).

CyMV on tools can be inactivated by heat and chemicals. Heat is the most effective inactivator. Since virus particles are largely protein, it can be precipitated by heating much like the white of an egg is when cooked. Bleach (Clorox, 5.75% Sodium hypochloride) has been found to be effective in concentrations as low as 2 to 4%. Saturated trisodium phosphate is also effective. Isopropanol alone did not completely inactivate CyMV even after a 10 min. soak. Ishii (1968) recommends a one min. soak in 4% bleach (Clorox) with frequent renewal of soaking

solutions. An alcohol dip followed by flaming was found to be equally effective (Ishii, 1968; Lawson, 1967).

Practices recommended to minimize CyMV infection in collections include:

- 1) Use of seedlings, as CyMV is not carried through the seed.
- 2) Selection of virus-free propagation stock.
- 3) Isolation or destruction of diseased plants.
- 4) Sterilization of cutting and propagation tools.
- 5) Use of fresh or sterilized pots, stakes, and media.
- 6) Control of insect pests which may be infecting plants.
- 7) Washing hands with soap and water before handling orchid plants.

Similar recommendations were presented by Jensen (1959), Kado (1964), Francki (1968) and Ishii and Martinez (1973).

An attempt by a private grower, Sporles (1983), to rid his orchid collection of CyMV and ORSV was fairly successful. He tested all plants (via serology) and rogued all virus infected plants from his collection. All incoming and new plants were screened in a similar manner. Strict sanitation procedures similar to those outlined previously were rigidly followed including the isolation and retesting of plants that did poorly. After four years, he retested all

plants (279) and found only 4.3% to be infected. He concluded that a virus-free collection was an attainable goal.

A breeding program for CyMV resistant strains has not yet been attempted. If CyMV resistant strains are available or can be produced, it would greatly reduce losses encountered by growers by this "inevitable" disease (Dunn, 1980).

III. MATERIALS AND METHODS

Plant Material

Dendrobium plants used in this study are a part of the collection of orchids housed at the Mauka Campus, University of Hawaii at Manoa. Three commercial cut flower cultivars were used; Uniwai Blush (UH44) released in 1972, Uniwai Supreme (UH232) released in 1976, and Uniwai Pearl (UH306) released in 1979 (Kamemoto, 1980). All are seed propagated amphidiploids.

Plants were grown in 1 gallon black plastic pots or 6-inch cement pots. Crushed blue rock (size no. 3) was used as media. Plants were sprayed weekly with insecticides and every two weeks with 20-20-20 foliar fertilizer. In the greenhouse, light intensity was approximately 3400 foot candles and temperatures ranged from 18°C to 33°C. In the saranhouse, light intensity was approximately 9600 foot candles and temperatures ranged from 15°C to 32°C.

Other Dendrobium species and hybrids used in the breeding program were also included in this study to observe differences in expression and progression of CyMV symptoms in flowers.

CyMV Indexing

A standard CyMV bioassay technique similar to that described by Lawson and Ali (1975) and demonstrated by Ishii (1982) was employed. This indexing method was

chosen because of its reliability (accuracy), low cost, and the ease of obtaining the necessary materials.

The indicator plant used was Cassia occidentalis (L.), a plant which responds to CyMV by producing local lesions. Plants were grown in one gallon plastic pots under greenhouse conditions. They were trimmed back as necessary to keep plants at a manageable size. Outbreaks of powdery mildew fungus (Oidium sp.) were controlled by application of methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) as recommended by Ishii (1973).

A young unblemished leaf was collected from each dendrobium plant. A 4 cm² sample was cut from each leaf and placed into a sterilized plastic mortar. Phosphate buffer (0.1M K₂HPO₄) was added to cover the sample and the tissue was ground with a glass pestle to release the cell contents.

The indicator plant (Cassia) was then prepared to receive the inoculum. A young, fully expanded compound leaf was chosen and two side leaflets were lightly dusted with 600 mesh carborundum powder (silicon carbide). The orchid sap extract was then gently rubbed over the dusted leaves using a cotton-tipped swab. The abrasive carborundum created small wounds which allowed the virus particles, if present, to enter the living cell. Excess powder and sap were then rinsed off the leaf. Plants were left in the greenhouse to incubate for 5 to 14 days. Inoculated leaves

were then examined for small brown necrotic spots which indicate the presence of CyMV. The number of spots per leaf indicating the severity of infection was recorded.

Degree of Infection in Relation to Age of Plants

Plants of different age groups of three commercial cut flower cultivars (UH44, UH232, UH306) were grown under either greenhouse or saranhouse conditions. These plants were indexed for CyMV presence to determine how CyMV infection is affected by time spent in cultivation.

Effect of CyMV on Yield

All groups of plants indexed in this study were previously used in various experiments and therefore have had flower yield data recorded for them. Two groups each of K232 (n=21, 22) and K44 (n=22, 22) were used in an experiment to determine the effects of environment on yield. One group of K610 (n=60) was used in a fertilizer experiment. Groups of plants of K603 (n=21), K564 (n=17) and K565 (n=19) have had yield data recorded for them. Comparing the yields of plants with and without CyMV was used to indicate the effect of CyMV on flower yield in these commercial dendrobium cultivars.

Expression of Necrosis in Flowers

Genetic variation in disease resistance or symptom expression is necessary if a breeding program for resistance

to flower necrosis caused by CyMV is to progress. Three commercial cultivars (UH44, UH232, UH306) were examined for correlation of CyMV symptom expression with CyMV infection as indicated by the bioassay testing.

Comparison of the onset and progression of the flower necrotic symptoms was used to determine if there are genetic differences in symptom expression. Various Dendrobium species and hybrids known to be infected with CyMV were examined for the onset and progression of necrotic symptoms in flower racemes. Flower racemes were recorded as to number of flowers and number of flowers displaying necrotic symptoms at 3 stages of maturity; when three quarters of the flowers were open, when all flowers were open, and at an old stage, 2 or more weeks after all flowers opened.

IV. RESULTS AND DISCUSSION

Degree of Infection in Relation to Age of Plants

The degree of CyMV infection was found to increase with time spent in cultivation. The correlation coefficient between age of plants and percent CyMV infection was 0.89 ($p=0.0006$). The percentage of plants infected ranged from a low of 19.0 for a 4 yr. 5 mo. old group of 'Uniwai Pearl' to 62.8 for a 6 yr. 4 mo. old group of 'Uniwai Supreme' (Table 1, Fig. 1). Since all plants used in this study were seed propagated, and CyMV is not transmitted through seed propagation in Dendrobium (Yuen et. al., 1979), it can be assumed that all infections occurred during the time the plants were in cultivation.

Surveys by Dunn (1980) and Sheehan et. al. (1981) indicated that plants that were in cultivation under 4 years showed little or no CyMV infection. CyMV infection rates increased dramatically after 4 years and can be expected to reach 100% in collections of plants greater than 10-15 years of age (Dunn, 1980; Sproles, 1983). Since the youngest plants surveyed in this study were slightly over 4 years old and had already incurred approximately 20% CyMV infection, it cannot be determined when the first infections occurred.

Seed-propagated Dendrobium cultivars, started from virus-free seeds spend the first 1-1 1/2 years isolated under aseptic conditions in flasks where there is

Table 1. Degree of infection of Dendrobium hybrids in relation to age of plants.

Cross No.	Cultivar	No. of Plants	Location	Age of Plants at Time of Indexing	Percent Infected with CyMV
K610	Uniwai Pearl	60	Saranhouse	4 yrs. 3 mos.	21.7
K603	Uniwai Pearl	21	Saranhouse	4 yrs. 5 mos.	19.0
K564	Uniwai Pearl	17	Saranhouse	4 yrs. 10 mos.	23.5
K565	Uniwai Pearl	19	Saranhouse	4 yrs. 10 mos.	31.6
K44	Uniwai Blush	22	Greenhouse	5 yrs. 0 mos.	36.4
K232	Uniwai Supreme	21	Greenhouse	5 yrs. 0 mos.	38.1
K44	Uniwai Blush	22	Saranhouse	5 yrs. 5 mos.	36.4
K232	Uniwai Supreme	22	Saranhouse	5 yrs. 5 mos.	50.0
K232	Uniwai Supreme	43	Greenhouse	6 yrs. 4 mos.	62.8
K232	Uniwai Supreme	44	Saranhouse	6 yrs. 10 mos.	52.3

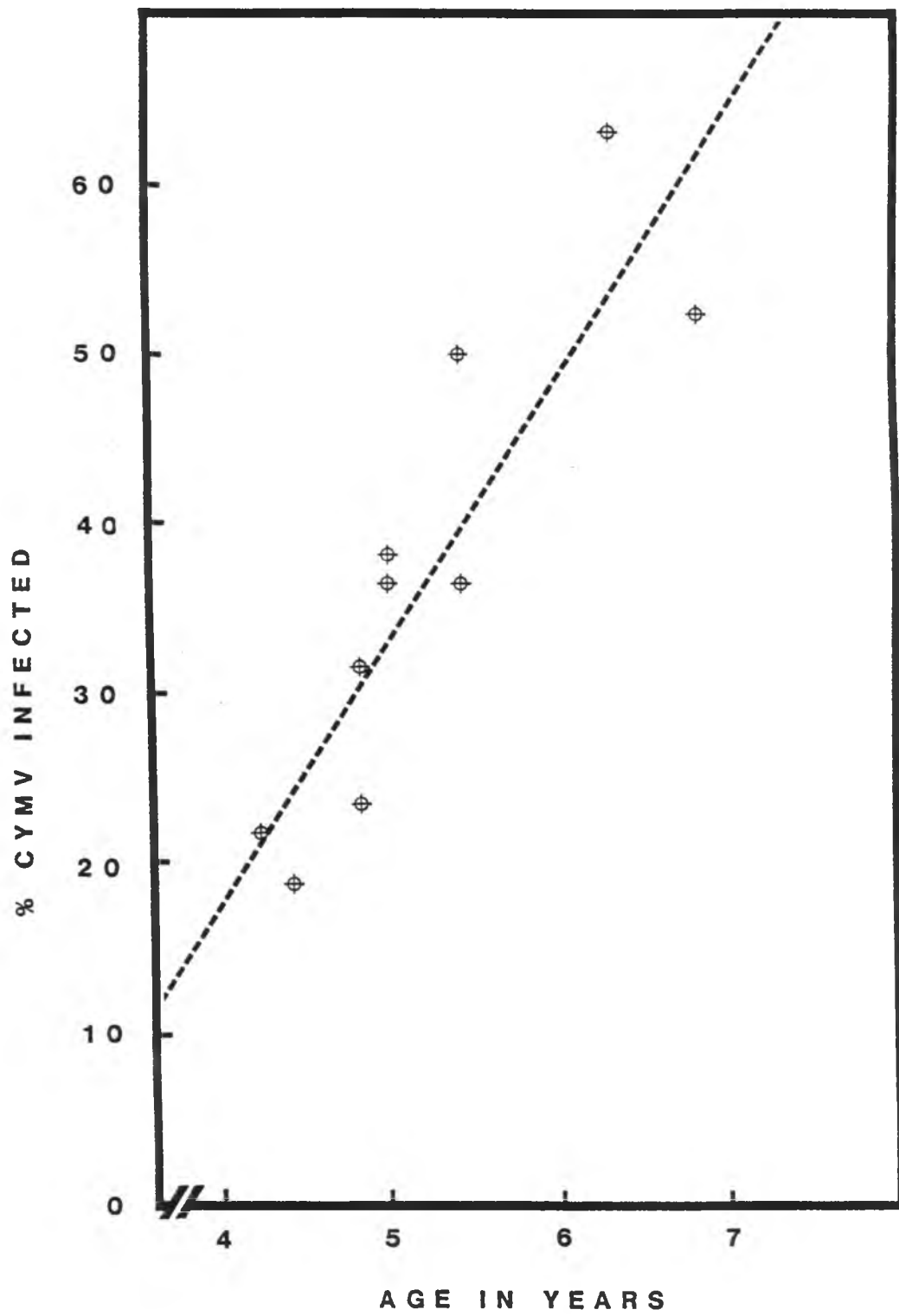


FIG. 1 - CYMV INFECTION RATES OF DENDRODIUM HYBRIDS VS AGE OF PLANTS.

practically no chance of CyMV contact or infection. The following year is spent in community pots. Plants at this stage are kept relatively isolated and are rarely handled. Although there is a greater potential for CyMV infection than in the flask, neighboring plants are usually other seedlings and therefore not likely to be infected and serve as CyMV reservoirs. Seedlings spend the following year in 2-inch pots where they are similarly isolated and rarely handled, thus posing little chance for infection. It is after this stage, and roughly coinciding with the 3-4 year age mark that the potential for CyMV infection dramatically increases. At this stage, they are transferred to 6-inch or 1 gallon pots. They are moved to greenhouse or saranhouse conditions with mature plants of widely varying ages and degrees of CyMV infection. At this stage, plants are larger and production of flowers begins. Both of these characteristics greatly enhance the potential of CyMV infection by increasing incidental contact with other plants and handling by researchers. In light of this, the 20% (approximate) infection rate incurred by the 4-year old plants in this study is consistent with expectations and would not be an unreasonable figure to expect in other collections, both commercial and hobbyist.

A regression analysis shows that after four years of age, CyMV infection increased 15.7% for every year in cultivation up to a maximum of 62.8% infection for a group

of UH232 at 6 yrs. 4 mos. of age (Table 1, Fig. 1). The equation for the regression line is $y=15.67x - 44.84$, and produces a good fit ($R^2=0.7857$, $p=0.0006$) to the data. If the observed trend continues, it is expected that CyMV infection percentages should continue to increase with increasing age of plants up to the maximum of 100% infection at somewhere between 10-15 years of age. This prediction is consistent with Dunn (1980) and Sproles (1983) who found collections of this age to be completely infected.

At an early stage of cultivation, only a few plants are infected, minimizing contact and spread to other plants. As infection percentages increase, contact is more extensive, making it easier to infect a larger number of plants. This might result in an exponential growth in infection percentages. The data however indicates a linear growth in infection percentages due possibly to the variation in treatments, crosses and sample size. Further testing, such as that of a single cross surveyed yearly for a period of 7-10 years, would greatly clarify the situation.

Minor discrepancies in the infection data might be expected, since CyMV is spread by cultural practices. The degree of handling and precautions taken will greatly affect infection rates. Sheehan et al. (1981) found considerable variation in percentage virus infections (17.5-54.4%) in 7 collections of cultivated orchids due in part to cultivation practices of the growers. Plants

in this study had previously been utilized in different research projects, and differ with respect to the amount of handling and precautions taken to minimize CyMV spread. For example, the group of Uniwai Supreme (6 yrs. 4 mos.) housed in the greenhouse displays a higher percentage of infection (Table 1) when compared with plants of a similar age. At the termination of a previous experiment in 1981, those plants were trimmed back severely, using shears that were not sterilized between cuttings (Kamemoto, 1983). This practice would greatly aid in the dispersal of CyMV and could account for the elevated infection percentage seen in this group.

Sample size between groups is highly variable ranging from $n=17$ to $n=60$. In small samples, a difference of infection of a single plant could account for as much as a 5-6% difference in percent infected. This may in part account for some of the variation observed.

Uniwai Blush (UH44) grown in the greenhouse which was first indexed in June, 1982 exhibited a 36.4% (8 of 22 plants) infection. A second survey approximately 11 months later in May, 1983, showed 3 additional plants (11 of 22 = 50%) to be infected with CyMV (Table 2). UH44 grown in the saranhouse which was first indexed in November, 1982, exhibited a 36.4% (8 of 22 plants) infection. A second survey approximately 6 months later in May, 1983, showed 2 additional plants (10 of 22 = 45.5%) to be infected with

Table 2. CyMV infection rates of UH44 grown in the greenhouse.

Plant No.	6/29/82		5/12/83	
	Infection ^Z	Lesions ^Y Per Leaf	Infection ^Z	Lesions ^Y Per Leaf
H29-9 (Control)	+	27, 17	+	10, 46
IA-1	+	70, 135	poor cond.	
-2	+	36, 29	+	22, 36
-3	-		-	
-4	-		-	
-5	-		-	
-6	+	95, 248	+	76, 110
-7	-		-	
-8	+	28, 78	+	41, 34
-9 ^x	-		+	5, 7
-10 ^x	-		+	12, 16
-11	-		-	
-12	-		-	
-13	+	93, 51	+	6, 11
-14	-		-	
-15	-		-	
-16	+	40, 34	+	118, 41
-17	-		-	
-18	+	9, 16	+	98, 93
-19	+	83, 90	+	70, 35
-20 ^x	-		+	28, 149
-21	-		-	
-22	-		-	
Percent Infected		8/22 = 36.4%		11/22 = 50%

^Z+ indicates plant infected with CyMV; - indicates plant not infected with CyMV.

^Y the two numerical values the number of local lesions on each of two leaflets of Cassia occidentalis used in the bioassay procedure.

^x plant changed infection status between surveys.

Table 3. CyMV infection rates of UH44 grown in the saranhouse.

Plant No.	11/16/82		5/6/83	
	Infection ^Z	Lesions ^Y Per Leaf	Infection ^Z	Lesions ^Y Per Leaf
H29-9 (Control)	+	72, 115	+	46, 118
IIA-1	-		-	
-2	-		-	
-3	-		-	
-4	+	29, 38	+	5, missing
-5 ^x	-		+	44, 53
-6	-		-	
-7	-		-	
-8	+	4, 3	+	9, 2
-9	+	142, 73	+	19, 26
-10 ^x	-		+	14, 37
-11	+	82, 166	+	63, 23
-12	-		-	
-13	-		-	
-14	-		-	
-15	-		-	
-16	+	5, 3	+	27, 22
-17	-		-	
-18	-		-	
-19	+	7, 15	+	5, 9
-20	+	3, 2	+	4, 2
-21	+	8, 4	+	2, 5
-22	-		-	
Percent Infected	8/22 = 36.4%		10/22 = 45.5%	

^{Z+} indicates plant infected with CyMV; - indicates plant not infected with CyMV.

^Y the two numerical values the number of local lesions on each of two leaflets of Cassia occidentalis used in the bioassay procedure.

^x plant changed infection status between surveys.

CyMV (Table 3). Presumably, the infections took place in the time period between surveys. This demonstrates the dynamic nature of the disease and the increase in infected plants over time. In all cases, no plant known to be infected with CyMV "escaped" detection in the second assay. This establishes the reliability of the bioassay indexing procedure.

The two locations, greenhouse and saranhouse, did not appear to affect CyMV infection rates. The 5-year old plantings of UH44 and UH232 in the greenhouse and saranhouse exhibit similar infection percentages (Table 1). UH44 plants grown in each location displayed identical 36.4% infection. UH232 plants grown in the greenhouse produced 38.1% infection while UH232 plants grown in the saranhouse displayed a high 50.0% infection rate. Location differences between the greenhouse and saranhouse are environmental differences such as light, temperature, precipitation, disease and insect pests. Since CyMV has no known insect vectors and its main agent of dispersal is the cultural practices of the grower, it is not surprising that location has little effect on CyMV infection levels.

Effect of CyMV on Flower Yield

The difference in mean flower spray yield per plant between CyMV-infected and non-infected plants were not statistically significant at the 5% level (Table 4). In six of the eight groups surveyed, CyMV-infected plants show slightly lower mean yields. For three groups; K564, K565 (both 'Uniwai Pearl'), and K232, the differences were significant at the 0.15 level. If a 0.15 confidence level is accepted, then the yields of CyMV-infected plants were significantly reduced in the above three groups. Although the differences may appear small (1.1 to 2.2 sprays/plant/year), it could translate into large economic differences at the commercial growers level. Commercial dendrobium growers plant approximately 30,000 plants per acre. A difference of 1 to 2 sprays/plant/year could mean a difference of 30,000 to 60,000 sprays per acre per year. Growers should be concerned about the possible economic consequences of CyMV.

The dynamic and elusive nature of the disease makes it difficult to obtain an accurate determination of the effects of CyMV on yield. Yield data collection was largely done before plants were indexed for CyMV. It is not known when the plants became inoculated with CyMV and therefore it is not possible to determine at what point in time flowers were being produced by an infected plant. CyMV is not

Table 4. Flower spray yields of CyMV infected and non-infected Dendrobium hybrids.

Cross No.	Location	Time Interval	<u>Total Sprays/Plant/Time Interval</u>		Probability Level ^z (F-Test)
			Infected	Non-infected	
K610	Saranhouse	8/81 to 12/82	6.7 \pm 6.69	7.3 \pm 3.07	0.91ns*
K603	Saranhouse	3/81 to 12/82	7.3 \pm 1.71	8.0 \pm 2.52	0.57ns
K564	Saranhouse	7/80 to 11/82	11.3 \pm 4.03	12.4 \pm 2.22	0.12ns
K565	Saranhouse	7/80 to 11/82	10.3 \pm 1.75	11.6 \pm 3.8	0.14ns
K44	Greenhouse	1/81 to 12/81	7.5 \pm 3.21	8.4 \pm 4.55	0.36ns
K232	Greenhouse	1/81 to 12/81	6.1 \pm 2.47	8.3 \pm 4.45	0.13ns
K44	Saranhouse	1/82 to 12/82	7.4 \pm 2.07	7.3 \pm 1.68	0.50ns
K232	Saranhouse	1/82 to 12/82	7.3 \pm 1.90	7.0 \pm 2.10	0.76ns

^z* ns indicates non-significance at the 5% level.

seed transmitted (Yuen et al., 1979) and therefore young seedlings are not likely to be infected. Much of the early yields are consequently produced by non-infected plants. As a result, yield data represented here are quite likely to include both non-infected and infected phases of flower production in a given plant. Early yield data may thus be acting as a buffer in the data set minimizing differences.

The principal method of CyMV dispersal is through mechanical transmission (Batchelor, 1982). Plants with higher natural flower yields receive a greater amount of handling during data-taking and harvesting. They therefore have more opportunity of coming into contact and becoming inoculated with CyMV. Since high yielding plants are more likely to be inoculated in this manner, it would counter the effect of any yield reduction that CyMV may cause. This may account for the non-significant differences in the yields of infected and non-infected plants.

Many factors, both genetic and environmental, affect yield. The perplexing nature of CyMV disease compounded by these factors make yield studies difficult to interpret. Chance inoculations, as surveyed here, may be selecting for high yielding plants. Also the time of inoculation cannot be determined for certain by the researcher. A better method would be to randomly place plants from a single cross into two groups and mechanically inoculate one of the groups at a single point in time. Plants should

be raised in identical conditions but separated to minimize the occurrence of chance infections of the non-infected group. Yields subsequently taken from such a design would better reflect the effect of CyMV.

Expression of Necrosis in Flowers

In all groups, the expression of necrotic symptoms in flowers was found to be highly correlated with infection with CyMV (Table 5). This result was expected because floral necrosis is one of the symptoms induced by CyMV infection (figures 2 to 7).

A small number of plants infected with CyMV did not display necrotic floral symptoms. This suggests that these plants may be resistant to CyMV symptom expression in flowers. The highest percentage of these resistant plants were found in the two groups of K232 ('Uniwai Supreme'), which produces a lavender and white bi-colored flower. Low percentages of resistant plants were found in all groups of K44 ('Uniwai Blush'), and K306 ('Uniwai Pearl') type crosses (K610, K603, K564, K565). These produce basically white flowers with K44 having a slight lavender tinge.

Lawson and Hearon (1973) found that white-flowered cattleyas infected with CyMV, particularly Cattleya Katherine Walker 'Whittie' and Cattleya 'Bow Bells', display prominent flower necrosis one to two weeks after the flowers open. In contrast, lavender-flowered cattleyas, particularly Slc. Falcon, failed to show flower necrosis even when infected with the same strain of the virus that produced extensive necrosis in susceptible flowers. In the present study, lavender flowered cultivar (K232) also produced the greatest

Table 5. Expression of necrotic symptoms in flowers in relation to CyMV infection.

Cross No.	n	CyMV/Floral Necrosis ^Z				Corr. Coef. ^Y
		+/nec.	+/no nec.	-/nec.	-/no nec.	
K610	60	11	2	2	45	0.80
K603	21	3	1	1	16	0.69
K564	17	4	0	3	10	0.66
K565	19	6	0	1	12	0.89
K44 ^X	22	8	3	1	10	0.65
K44 ^X	22	9	1	2	10	0.73
K232	22	8	3	0	11	0.76
K232	44	15	8	3	18	0.52

^Z+ indicates CyMV infection; - indicates non-infection; nec. indicates necrotic floral symptom expression; no nec. indicates no necrotic floral symptoms.

^Y all correlation coefficients are highly significant.

^X includes data from 5/12/83 indexing.



Figure 2. Front view of necrotic flower.

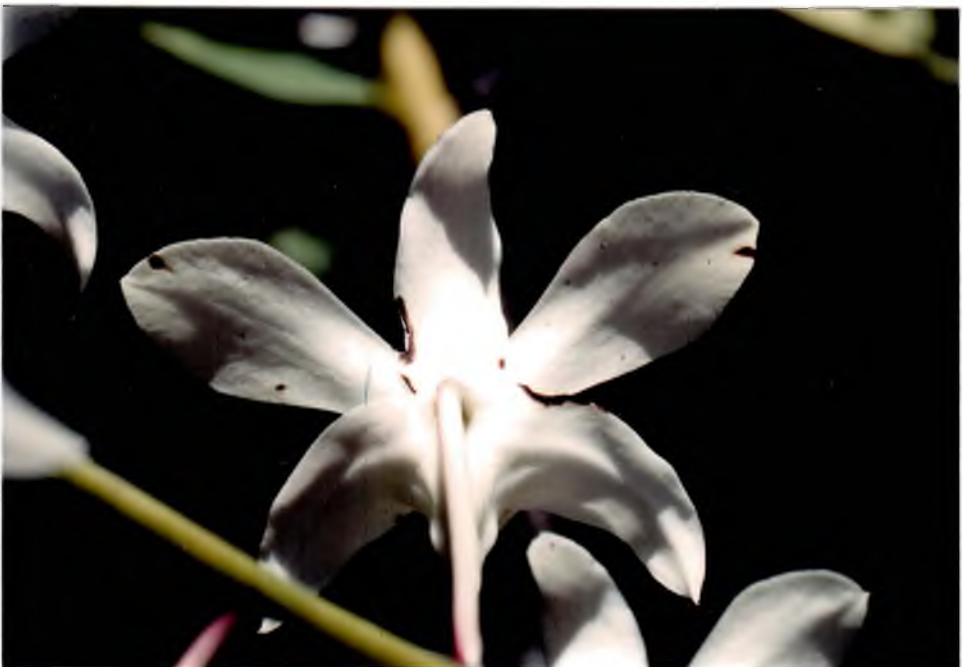


Figure 3. Rear view of necrotic flower.

Figures 2-3. CyMV-induced flower necrosis on Dendrobium Jaquelyn Thomas 'Uniwai Blush', (UH44).



Figure 4. Front view of necrotic flower.



Figure 5. Rear view of necrotic flower.

Figures 4-5. CyMV-induced flower necrosis on Dendrobium Jaquelyn Thomas 'Uniwai Supreme', (UH232).



Figure 6. Front view of necrotic flower.



Figure 7. Spray containing necrotic flowers.

Figures 6-7. CyMV-induced flower necrosis on Dendrobium Jaq-Hawaii 'Uniwai Pearl', (UH306).

number of resistant plants while the white-flowered cultivars (K44, K306) showed little resistance. This may indicate a relationship between some factors of color expression and resistance.

A small number of non-infected plants in each group was found to display necrotic symptoms in flowers. This result was not expected and is incompatible with the concept of CyMV-induced floral necrosis. Some groups of plants were tested for CyMV as much as 11 months prior to the final survey for floral necrosis in early June, 1983. It is possible that plants in this category were inoculated with CyMV during this time period and were reflecting true CyMV-induced necrotic symptoms. Alternately, CyMV-infected plants may have escaped detection by the indexing procedure. However, the bioassay procedure was highly reliable. All CyMV-infected controls and infected plants that were re-tested (Tables 2 and 3) were consistently indicated to be positive for CyMV. Therefore, few, if any, CyMV-infected plants are likely to have escaped detection by this procedure. Other causes such as fungal infection, sunburn, spray injury or nutritional imbalances may have produced virus-like symptoms in non-infected plants. However, since CyMV-induced floral necrotic symptoms are specific, misdiagnosis is not likely. In any case, suspect plants in collections should be retested if virus is suspected.

CyMV symptom severity, as indicated by the number of necrotic flowers per spray, increases with flower maturity (Table 6). A large amount of variation in both the rate and severity of expression of necrotic flower symptoms occurs between plants of different crosses. Plants such as D40, D40-1C, K133-1 and K135-2 remained completely symptomless in all sprays. D40 and D40-1C are D. phalaenopsis 'Kosaki' while K133-1 is an selfed offspring of D40-1C. On the other extreme, plants such as K339-17, K339-19, TR-1, TR-2, H29-9, H29-30, and H29-34 show extensive flower necrosis at the harvest stage (3/4 bloom). Necrotic symptom expression of flowers increased to nearly 100% by the time all flowers on the spray were open. Crosses producing the most extensive symptoms have D. phalaenopsis 'Lyons Light no. 1' and/ or 'Lyons Light no. 2' in their background, plants that are highly susceptible to necrotic expression of flowers (Fig. 8).

Similar behavior in pattern and severity of CyMV-induced flower necrosis in genetically related plants are consistently observed. Since all plants were grown under identical greenhouse conditions, this suggest a genetic component to differences in expression. This type of variation is necessary if a breeding program for resistance to CyMV-induced necrotic flower symptoms is to progress. These data offer evidence that resistant genes are present in commercial dendrobium lines which could be used to increase resistance of dendrobium cultivars to CyMV.

Table 6. Expression of necrotic symptoms at different stages of spray maturity.

Plant No.	No. Sprays Examined	Mean Flowers Per Spray	Mean % Necrotic Flowers Per Spray ^z		
			3/4	1	Old
D40	6	9.2 + 2.7	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0
D40-1C	2	6.5 + 0.7	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0
D47-2	11	14.5 + 3.5	28.1 + 14.0	67.2 + 7.8	95.9 + 5.4
Y166-1	4	16.0 + 5.4	0.0 + 0.0	15.1 + 21.0	71.7 + 32.8
K133-1	4	4.8 + 1.3	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0
K135-2	3	6.7 + 2.3	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0
K339-17	11	11.4 + 2.9	38.5 + 16.6	94.2 + 7.1	99.1 + 3.0
K339-19	6	14.0 + 2.5	4.8 + 11.7	66.8 + 28.1	99.0 + 2.4
K350-1	2	7.5 + 3.6	0.0 + 0.0	0.0 + 0.0	25.0 + 35.4
K350-3	4	13.5 + 6.6	6.0 + 11.9	50.6 + 17.9	89.7 + 20.6
K384-30	20	17.1 + 3.5	7.2 + 15.5	18.4 + 28.0	44.6 + 35.4
K384-30B	9	18.9 + 2.0	16.5 + 12.0	32.9 + 22.9	52.4 + 30.4
TR-1	10	9.7 + 1.8	79.2 + 12.8	99.0 + 3.2	100.0 + 0.0
TR-2	2	4.5 + 0.7	0.0 + 0.0	100.0 + 0.0	100.0 + 0.0
H29-9	5	13.8 + 4.0	60.0 + 24.5	98.6 + 3.2	100.0 + 0.0
H29-30	5	14.2 + 1.9	29.2 + 6.6	50.0 + 11.1	97.2 + 3.8
H29-34	2	15.5 + 3.5	33.3 + 0.0	45.3 + 1.2	62.4 + 9.7
8-14	2	9.5 + 2.1	0.0 + 0.0	55.1 + 24.9	95.5 + 6.4
1-15	3	19.0 + 7.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0

^z 3/4 indicates spray with 3/4 of its flowers open, normal stage for harvest; 1 indicates a mature spray with all of its flowers open; old indicates an old spray, 2 or more weeks after all flowers have fully opened.



Figure 8. Extensive necrosis in flower spray of H29-9, Dendrobium phalaenopsis 'Lyons Light no. 1' X 'Lyons Light no. 2'.

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