mg ancymidol as a drench or granular material but plants receiving a foliar spray at the same concentration were similar in height to controls. Other researchers (4) obtained height reduction of 'Nob Hill' with a foliar spray application of ancymidol with substantially greater concentrations (5000 mg/liter). The present results suggest ancymidol at this concentration is either absorbed more effectively through roots than leaves or the chemical was absorbed through the leaf but is not translocated basipetally to inhibit gibberellin synthesis in roots (7).

'Royal Trophy' was more responsive to growth regulator treatments than 'Yellow Mandalay'. Daminozide spray reduced growth of 'Royal Trophy' 46% while 'Yellow Mandalay' was 27% shorter than untreated plants. Similar responses were observed on plants grown in media with granular ancymidol. Differential response of cultivars to growth regulators has been reported previously (4,6).

Flowering was hastened 5 days on 'Royal Trophy' treated with 0.5 mg ancymidol soil drench. Other treatments did not delay flowering of this cultivar. All growth regulator treatments slightly delayed flowering of 'Yellow Mandalay'. Flower number was reduced on 'Yellow Mandalay' with 0.25, 0.5 and 1.0 mg of granular ancymidol and ancymidol drench and on 'Royal Trophy' with all treatments except the lowest rate of granular ancymidol.

Although granular ancymidol is not as effective on chrysanthemums as poinsettia (12), these findings suggest granular ancymidol incorporated into the growing medium is an effective application method and could reduce labor required to apply growth regulating chemicals. Additonal research is needed to identify responsive cultivars and the influence of environment.

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A Rapid Method for Virus-indexing the Florist's Geranium[1]

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Additional index words. enzyme-linked immunosorbent assay (ELISA), *Pelargonium* X *hortorum, tobacco ringspot virus*

Abstract. **The enzyme-linked immunosorbent assay (ELISA) was used for the detection of tobacco ringspot virus (TRSV) in crude leaf extracts of geranium (*Pelargonium* × *hortorum* Bailey). An ELISA test protocol using a coating antibody concentration of 10 μg/ml, a 1000-fold dilution of antibody-enzyme conjugate, a substrate reaction time of 30-45 minutes, and a leaf test sample prepared in 20 volumes of phosphate buffer provided the best compromise between test reliability, conservation of reagents, and convenience. Using this protocol, significantly positive absorbance values (A405nm) were obtained with extracts from geraniums latently-infected with TRSV whereas color development form noninfected geraniums was negligible. Potentially, the ELISA represents a rapid, reliable, economical, and convenient method for virus-indexing geraniums commercially.**

Numerous virus diseases of florist's geranium have been described, but that incited by tobacco ringspot virus (TRSV) is probably the most widespread and destructive (5, 6). Since this disease is characterized by the occurrence of a latent (symptomless) phase, inspection of plants for visible symptoms does not provide a reliable basis for detecting virus infection. Consequently, an infectivity bioassay is used for indexing geraniums in commercial production for TRSV and several other economically important viruses (8). In this procedure, an extract from a geranium plant is rubbed onto a series of indicator plants which, when infected with virus, develop obvious symptoms. However, a potent inhibitor in geranium leaf extracts drastically reduces the sensitivity of this test which, in turn, has limited the effectiveness of this certified stock program (9). In recent years, a serologic test known as the enzyme-linked immunosorbent assay (ELISA) has gained widespread application for the detection of latent viruses which occur in plant species whose extracts contain inhibitors of viral infection (2, 3). We now present evidence that antiserum to TRSV can be used in the ELISA for the detection of TRSV in crude extracts of geranium. We also describe some parameters which optimize the reliability of the test.

A geranium isolate of TRSV was partially-purified by the procedure described by Steere (10), and further purified by 2 cycles of sucrose density gradient centrifugation. Antibodies to TRSV were induced in 'New Zealand White' rabbits by repeated intradermal injections. The antibodies were then purified and conjugated to alkaline phosphatase following the methods of Clark and Adams (4). Briefly, the procedure for the microplate ELISA test entailed: coating the microplate well with TRSV antibodies; incubating a geranium leaf extract in the well; further incubating with antibody-enzyme conjugate; and finally, adding the enzyme substrate (4). The antibody-enzyme conjugate was diluted in PBS-T-PVP (0.02 M phosphate, 0.15 M NaCl, pH 7.4 with 0.05% Tween-20 and 2% polyvinyl pyrrolidone, MW 10,000) containing 0.2% ovalbumin to reduce nonspecific color development (7).

The seedling geranium cultivar 'Nittany Lion Red' was used exclusively

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in this study. Geraniums latently-infected with TRSV at 5 weeks after inoculation served as a source of infected material. Healthy controls consisted of leaf samples from noninfected plants of the same age. ELISA test samples were prepared by homogenizing leaves in PBS-T-PVP with a mortar and pestle, and filtering the extract through a layer of Miracloth.

Color development (virus concentration) was quantified by measuring absorbance at 405 nm (A_{405nm}^{1mm}) with a Beckman Model 3600 spectrophometer. A buffer control consisting of PBS-T-PVP substituted for a leaf extract as the test sample was included in every experiment. Data are expressed as net absorbance values calculated by subtracting mean buffer control value from mean treatment absorbance values.

Influence of the coating antibody concentration and antibody-enzyme conjugate dilution. ELISA test sensitivity was markedly affected by the coating antibody concentration and the antibody-enzyme conjugate dilution (Fig. 1). Absorbance values decreased with either a decrease in the concentration of the coating antibody or with an increase in the dilution of the conjugate. Using incubation conditions of 37°C for 3 hr, a coating antibody concentration of 10 μg/ml and a 1000-fold dilution of the conjugate afforded the greatest differential (15-fold) between the nonspecific color development of the healthy control plants and the specific reactions of the TRSV-infected plants. Consequently, these concentrations were adopted as the standard test protocol because they also offered the best compromise between test reliability and the conservation of reagents.

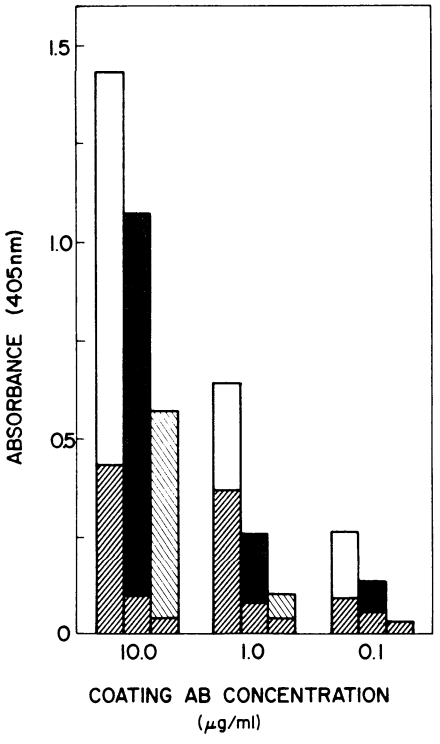

Fig. 1. Absorbance at 405 nm as a function of a 1/100 (□), 1/200 (■) and a 1/1000 (▨) dilution of antibody-enzyme conjugate and three concentrations of coating antibody using extracts from geraniums latently-infected with TRSV. Incubation conditions were 37°C for 3 hr. Absorbance due to noninfected controls (▨). Data points are the mean of 4 observations.

Influence of the substrate reaction time. The intensity of the specific reactions was also affected by the length of time the enzyme was allowed to react on the substrate (Fig. 2). Absorbance increased linearly with an increase in the substrate reaction time up to 1 hr at which time enzyme-substrate saturation levels were reached. Color development due to nonspecific reactions associated with healthy controls was low at each of the time intervals tested, although a slight increase in absorbance was observed after two hours. The difference between the specific and nonspecific reactions became maximal after 2 hr; however a reaction time of 30 to 45 min was routinely used for convenience because it provided adequate test sensitivity.

Influence of the extract dilution. When geranium leaves were homogenized in PBS-T-PVP buffer ranging from 3 to 100 volumes per weight of tissue, significantly positive reactions were always obtained with extracts from latently-infected plants (Fig. 3). The greatest differential between specific and nonspecific color development occurred when leaves were homogenized in a 10 to 20-fold excess of buffer.

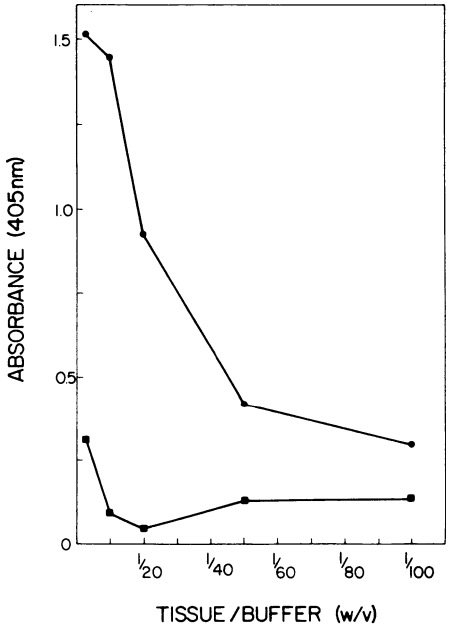

Fig. 2. The effect of substrate reaction time on the ELISA values (A405 nm) using extracts from noninfected geraniums (■-■) and geraniums latently-infected with TRSV (●-●). Data points are the mean of 4 observations.

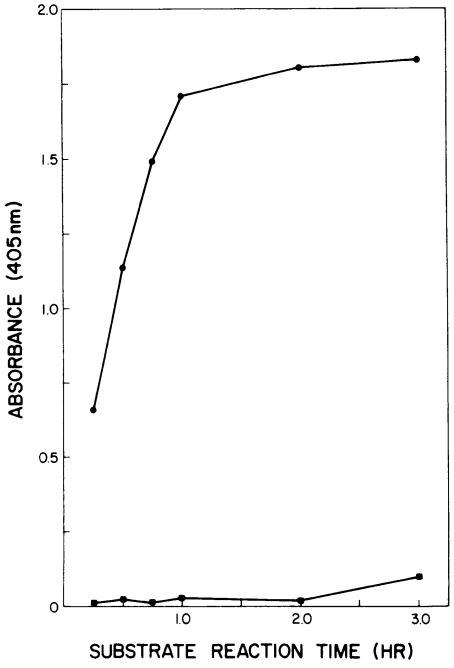

Fig. 3. The effect of the tissue to buffer ratio on the ELISA values (A405 nm) using extracts from noninfected geraniums (■-■) and geraniums latently-infected with TRSV (●-●). Data points are the mean of 4 observations.

Nonspecific reactions were highest at the lowest tissue to buffer ratio. Also, we have observed with other geranium cultivars (data not shown) that the geranium inhibitor is most refractory to the antibody-virus reaction at a tissue to buffer ratio of 1:3. This was not observed with the cultivar 'Nittany Lion Red' and we suspect that this reflects a variation between cultivars in the foliar content of the inhibitor. Consequently, a 1:20 tissue to buffer ratio was adopted as the standard test protocol because it provided a greater test reliability by minimizing both the influence of the inhibitor and the dilution of virus in the extract.

Our results show that the ELISA can be used to detect TRSV in crude geranium leaf extracts despite the presence of a potent inhibitor of the serologic reaction (1). Further, the ELISA test is sufficiently sensitive to detect TRSV in the latent stage of the disease. This is of paramount importance because diseased plants encountered in commercial production are most frequently latently-infected. Potentially, we feel that the ELISA could be implemented at the commercial level to vastly simplify and reduce the cost of virus-indexing geraniums in the certified stock program.

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Dikegulac Sodium Influences Shoot Growth of Greenhouse Azaleas[1]

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Additional index words. growth regulators, chemical pinching, apical dominance, *Rhododendron* spp.

Abstract. **Foliar sprays of 0.5% dikegulac sodium applied to 4 cultivars of greenhouse-forcing azaleas (*Rhododendron* spp.) 11 days after shearing, decreased shoot length and increased shoot number with more shoots originating along the entire stem at lower node positions than on untreated plants. Five to 6 weeks after treatment shoot length increased normally indicating that dikegulac sodium did not have a long term depressive effect on azalea shoot growth and development.**

Dikegulac sodium, the sodium salt of 2,3:4,5-bis-0-(1-methylethylidene-α-L xylo-2-hexulofuranosonic acid), has been extensively tested as a pinching agent on *Rhododendron* (3,4,6,10,11). Researchers reported that dikegulac sodium sprays destroy apical dominance and induce the production of axillary shoots (1,3). Dikegulac sodium has been found to be translocated to the plant apex (2) and to have an inhibitory effect on RNA (7) and DNA (2) synthesis and internodal expansion (2). Delayed plant growth (4,8,11), as well as retardation (3,8,11), has raised serious questions concerning the use dikegulac sodium in the production of *Rhodendron*.

Heursel (9) has reported that the growth delay might last 6 to 24 weeks depending on the number of applications, plant metabolism and environmental conditions. Cohen (5) noted that dikegulac sodium had no effect on *Rhododendron* shoot length 7 weeks after application. The purpose of the present work was to define the effect of dikegulac sodium on vegetative shoot growth of greenhouse azaleas.

Plants of the azaleas 'Kingfisher', 25 × 25 cm, and 'Alaska', Dorothy Gish', and 'Red Wing', 15 × 20 cm, were planted in 15 × 11 cm clay pots containing Canadian sphagnum peatmoss amended with 1.48 kg/m^3 each of dolomitic limestone and gypsum. About 120–180 ml of a fertilizer solution containing 2.5 g/liter of 25 N–4.4 P–8.2 K soluble fertilizer were applied to the medium of each pot every 2 weeks. Iron sulfate (1.0 g/liter) was added to the fertilizer solution to avoid iron deficiency. Plants were grown in a glass greenhouse with a maximum light intensity of 48.5 klx (measured at noon on a bright day). This photoperiod was supplemented during the night starting 2 weeks after treatment by using constant light from incandescent bulbs (208 lux at the top of plants) from 10 PM to 2 AM. Plants were sheared on December 23, 1978. A 0.5% dikegulac sodium spray was applied by a low pressure, high volume sprayer to runoff on sheared plants on January 3, 1979, for comparison with untreated sheared plants. A randomized complete block design was used with 7 replications, 3 plants per treatment (subsample) on 'Kingfisher' and 3 replications, 4 plants per treatment (subsample) on 'Alaska', 'Dorothy Gish' and 'Red Wing'. Two branches were chosen at random from each plant on which length of emerging shoots was measured at various node positions (counting basipetally) on January 31, February 7 and February 14, 1979. Total shoot number of each plant was determined on February 14.

Three to 4 weeks after treatment, newly developing leaves of dikegulac sodium-treated plants exhibited the necrotic leaf tip and chlorosis reported by other workers (1,4,6,7,11). The chlorosis disappeared in 6 to 8 weeks. It is suggested that this characteristic chlorosis may serve as an activity indicator of dikegulac sodium.

Four weeks after treatment, dikegulac sodium-treated plants produced new shoots at node positions 1 to 6 on 'Alaska', 1 to 8 on 'Kingfisher', and 1 to 9 on 'Dorothy Gish' and 'Red Wing' (Table 1). Untreated plants originated new shoots at node positions 1 to 4 ('Alaska'), 1 to 5 ('Red Wing') and 1 to 6 ('Kingfisher' and 'Dorothy Gish'). The average number of nodes per branch which produced shoots from dikegulac sodium-treated plants was 4.0 ('Alaska'), 5.1 ('Red Wing') and 5.2 ('Kingfisher' and 'Dorothy Gish') (Table 2). However, shoot emergence from the nodes on untreated branches averaged 3.1 ('Alaska'), 3.3 ('Kingfisher'), 3.5 ('Red Wing') and 3.6 ('Dorothy Gish'). The mean number of nodes producing shoots on dikegulac sodium-treated plants exceeded that of check plants for 'Alaska', 'Kingfisher' and 'Red Wing' but not for 'Dorothy Gish'.

New shoots were initially shorter on dikegulac sodium-treated plants than on check plants (Table 2). However, after 4 to 5 weeks, there were no differences in shoot length increases between treated and untreated plants except on 'Kingfisher'. At the 5 to 6 week interval, shoot length increases on all cultivars of dikegulac sodium-treated plants exceeded those of untreated plants. This suggested that dikegulac sodium did not a strong depressive effect on shoot growth 6 weeks after treatment.

New shoot length varied by node position (Table 1). Four weeks after treatment uniform length shoots were produced from nodes 1 to 4 on 'Alaska', 2 to 4 on 'Kingfisher', 1 to 5 on 'Red Wing' and 1, 2, 4, and 5 on 'Dorothy Gish'; while the check plants only produced uniform shoots from nodes 1 to 2 on 'Alaska', 'Kingfisher', and 'Red Wing'. Shoot length increased rapidly on treated 'Alaska' plants at 5 and 6 weeks and it was not as uniform on these plants as at 4 weeks. In contrast, shoot development remained uniform from nodes 1 to 3 on check plants. Six weeks after treatment shoot lengths of 'Dorothy Gish' plants were of uniform length at nodes, 2, 4, and 5 as well as nodes 1, 5, and 6.

However, check plants produced different shoot lengths at every node. Shoots developing from the first node

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