of illuminating fruit in storage.


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# Pathogenicity, Growth, and Sporulation of *Mucor mucedo* and *Botrytis cinerea* in Cold or CA Storage


**Andres A. Reyes**[1]

*Agriculture Canada Research Station, Vineland Station, Ontario L0R 2E0, Canada*



*Additional index words.* postharvest diseases, mucor rot, gray mold, vegetables



***Abstract.*** **The virulence of *Mucor mucedo* (L.) Fr. (the cause of mucor rot) and *Botrytis cinerea* Pers. (gray mold) on vegetables stored at 13C for 7 days or 1C for 70 days varied with the host and controlled atmosphere (CA). *M. mucedo* was severely pathogenic at 13C to cucumber *(Cucumis sativus* L.), eggplant *(Solarium melongena* L. var. *esculentum* Nees), pepper *(Capsicum annum* L.), and tomato *(Lycopersicon esculentum* Mill.), but not to bean *(Phaseolus vulgaris* L.). The fungus did not infect carrot *(Daucus carota* L. var. *sativa* DC.), celery *(Apium graveolens* L. var. *dulce* DC.), onion *(Allium cepa* L.), or parsnip *(Pastinaca sativa* L.) at 1C. *B. cinerea* was virulent on all of these crops at 13 or 1C. The severity of mucor rot and gray mold on eggplant stored at 13C for 14 days was suppressed most in a CA of 7.5% CO + 1.5% $O_2$ and least in 1.5% $0_2$, in comparison with the air control. Similarly, the growth and sporulation of each pathogen on eggplant-extract agar maintained at 13C for 4 or 14 days were suppressed most in 7.5% CO + 1.5% $O_2$; suppression was least** in 1.5% $O_2$. **The suppression of diseases on eggplant was highly correlated with the suppression of mycelial growth and sporulation of pathogens on agar.**


Of the numerous reports of *Mucor* spp. causing decay of vegetables in cold storage, *Mucor mucedo* has not been listed as a pathogen in North America (Weimer and Harter, 1921; Butler, 1959; Smith et al., 1979). In 1981, in the United States, Moline and Millner (1981) first observed that *M. mucedo* caused a severe decay (hereafter referred to as mucor rot) of tomato fruit within 3 to 4 days after inoculation at 20C. There is little information regarding the suppression of mucor rot in controlled atmosphere (CA). In contrast, *Botrytis cinerea* has been reported frequently to cause gray mold on stored vegetables in Europe or North America (Smith et al., 1966; Adair, 1971; Geeson and Browne, 1980; Geeson et al., 1988). Gray mold was found to cause decay of vegetables shipped to the New York markets (Cappellini et al., 1987; Ceponis et al., 1985, 1986, 1987a, 1987b, 1988). In Ontario, Canada, Reyes and Smith (1986) have shown that *B. cinerea* caused severe gray mold on stored celery and that the disease was suppressed by CA storage at 0 to 1C. Other studies have indicated that CA suppresses gray mold on cabbage (Adair, 1971; Geeson and Browne, 1980). The objectives of the present study were to 1) compare the pathogenicity of *M. mucedo* with that of *B cinerea* on vegetables in storage, and 2) determine if CA suppresses these diseases on eggplant and reduces growth and sporulation of the pathogens on agar.

The vegetables used in this study were 2-week-old (from anthesis), 10-cm-long, green bean pods *(Phaseolus vulgaris* L. c.w. Speculator); 8-week-old (from seeding), 4-cm-diameter carrot roots *(Daucus carota* L. var. *sativa* DC. cv. Spartan); 15-week-old (from seeding), 30-cm-long celery petioles *(Apium graveolens* L. var. *dulce* DC. cv. Tendercrisp); 2-week-old (from anthesis), 5-cm-diameter, green cucumber fruits *(Cucumis sativus* L. long type cv. Corona); 2-week-old (from anthesis), 5-cm-diameter eggplant fruits *(Solarium melongena* L. var. *esculentum* Nees narrow type cv. Tycoon); field-

Table 1. Pathogenicity of *Mucor mucedo* and *Botrytis cinerea* as indicated by average diameter of 10 replicate lesions on various vegetables stored at 1 or 13C for 70 and 7 days, respectively.

| | Lesion diam (cm)[z,y] | | | |
|---|---|---|---|---|
| | 1C | | 13C | |
| Vegetable | *Mucor mucedo* | *Botrytis cinerea* | *Mucor mucedo* | *Botrytis cinerea* |
| Bean | --- | --- | 0.0 f | 4.0 cd |
| Carrot | 0.0 b | 4.0 a | 0.0 f | 1.2 e |
| Celery | 0.0 b | 3.4 a | 0.0 f | 5.7 b |
| Cucumber | --- | --- | 5.0 bc | 5.4 b |
| Eggplant | --- | --- | 8.5 a | 5.8 b |
| Onion | 0.0 b | 3.0 a | 0.0 f | 1.2 e |
| Parsnip | 0.0 b | 3.6 a | --- | --- |
| Pepper | --- | --- | 4.5 c | 3.1 d |
| Tomato | --- | --- | 8.8 a | 3.6 d |

[z]Means within each column followed by the same letter are not significantly different (Duncan's multiple range test, $P = 0.05$).
[y]Dashes = no data collected.


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[1]Research Scientist.

dry (13 weeks from seeding), 6-cm-diameter onion bulbs *(Allium cepa* L. cv. Tamarack); 17-week-old (from seeding), 5-cm-diameter parsnip roots *(Pastinaca sativa* L. cv. Harris Model); 6-cm-diameter, mature, green pepper fruits *(Capsicum annum* L. cv. Plutona); and 6-cm-diameter, mature, green tomato fruits *(Lycopersicon esculentum* Mill. cv. Caruso). These vegetables were field-grown, except cucumber, pepper, and tomato, which were raised in a greenhouse. Before use, the vegetables were washed carefully in running tap water. Celery was prepared as described previously (Reyes, 1988).

The pathogens were a tomato isolate of *M. mucedo* (ATCC 48559) and a celery isolate of *B. cinerea* (Reyes and Smith, 1986). The mycelial inoculum was prepared by aseptically excising mycelial plugs (8 mm in diameter) from the margin of cultures grown on potato dextrose agar (PDA) (10 × 1.5-cm plates, 2.5% w/v) maintained at 22C for 3 days.

Mixed gases in 6-$m^3$ cylinders were prepared by Canadian Air (Montreal, Quebec) as follows: 1) 1.5% $O_2$, 2) 4% $CO_2$ + 1.5% $O_2$, 3) 7.5% CO + 1.5% $O_2$, and 4) 21% $O_2$ (air control), with the balance in each gas mixture as $N_2$. These gases were selected on the basis of a previous study (Reyes and Smith, 1986).

Table 2. Suppression of mucor rot and gray mold on eggplant stored in various atmospheres at 13C for 7 days.

| Atmosphere (%) | | | Suppression (%)[z,y] | |
|---|---|---|---|---|
| O $_2$C | $O_2$ | CO | Mucor rot | Gray mold |
| 21.0 (air control) | | | 0.0 d | 0.0 d |
| 1.5 | 0 | 0 | 22.7 C | 43.8 b |
| 1.5 | 4.0 | 0 | 14.8 C | 53.0 b |
| 1.5 | 0 | 7.5 | 11.7 c | 79.1 a |

[z]Disease suppression (%) was calculated as follows: lesion diameter in the air (control) minus lesion-diameter in the atmosphere divided by lesion diameter in the control multiplied by 100%. Mean lesion diameters in the control for mucor rot and gray mold were 8.5 and 4.2 cm, respectively.
[y]Means followed by the same letter are not significantly different *(t* test, $P = 0.05$).

To compare the pathogenicity of *M. mucedo* and *B. cinerea,* 10 replicates of each vegetable were placed in a plastic tray (54 × 25 × 12 cm)lined with moistened paper towels. Each vegetable was wounded (5 mm deep) in the middle with a l-mm-wide wooden toothpick, then a mycelial plug of *M. mucedo* or *B. cinerea* was aseptically placed over the wound. A tray of each vegetable receiving a plug of PDA without pathogen served as a noninoculated control. Then a similar plastic tray was inverted over each tray of vegetable. One tray each of carrot, celery, onion, and parsnip was stored at 1C, and another of each was stored at 13C. One tray each of chilling-sensitive bean, cucumber, eggplant, pepper, and tomato was stored at 13C only. The cross-diameters of each lesion were measured and averaged after 70 days of storage at 1C or after 7 days at 13C.

To determine the effect of CA on the severity of mucor rot and gray mold, 10 replicates of freshly harvested eggplants were placed in each of four trays, inoculated with *M. mucedo* or *B. cinerea,* and the trays covered as above. Then each tray was placed inside a low-density polyethylene (LDPE) bag (80 × 55 cm, 65 p. thick; Novacor Chemicals, Alberta, Canada) that was sealed, emptied of air under a vacuum, and filled with 40 liters of prepared gas mixture (Reyes, 1988). 'The permeability coefficients of the LDPE bags have been reported elsewhere (Reyes, 1988). To minimize the interference from any $CO_2$, ethylene, and other volatiles emitted by the vegetables or pathogens in the bags, the gas in each bag was removed by applying a vacuum, and each bag was refilled with a fresh supply daily. The gas composition of each bag was not measured at the end of the 24 hr. All bags were maintained at 13C, and the cross-diameters of each lesion were measured and averaged after 7 days. Disease suppression (percent) was calculated as follows: diameter of lesion in air (control) minus diameter of lesion in CA divided by the diameter of lesion in the control multiplied by 100%.

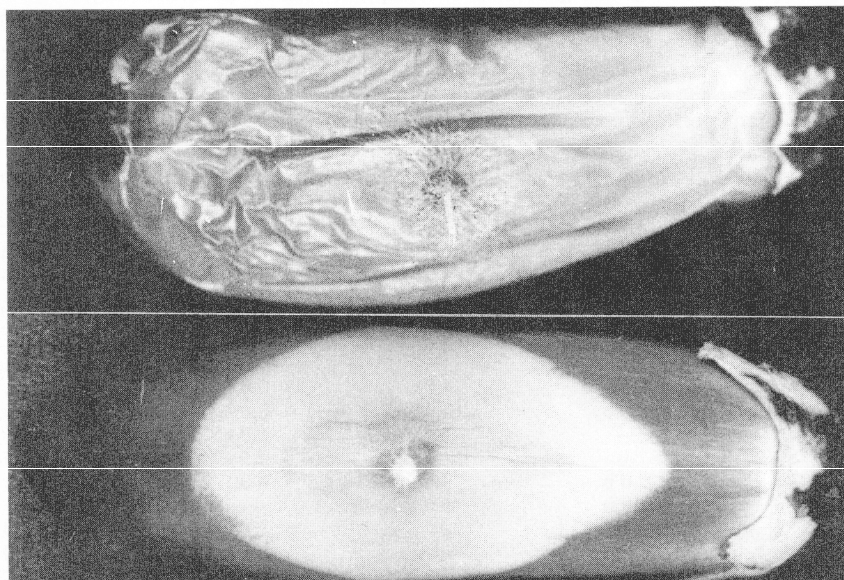

Fig. 1. Mucor rot caused by *Mucor mucedo* **(top)** and gray mold caused by *Botrytis cinerea* **(bottom)** on 'Tycoon' eggplant 7 days after inoculation at 13C.

To study the effect of CA on the mycelial growth and sporulation of *M. mucedo* and *B. cinerea,* a mycelial plug of each pathogen was transferred aseptically to the center of each of 40 plates of eggplant-extract agar (EEA) (2 g of Difco agar and 30 g of eggplant boiled for 25 min in 100 ml of distilled water and filtered). Ten replicate plates of each pathogen were placed in a LDPE bag. The bags were sealed, emptied of air, and filled separately with each of the four gas mixtures mentioned above. Each bag was emptied of gas and refilled with a fresh supply daily. On day 4, the plates were removed from the bags and the cross-diameters and spore content of each colony were determined. Plates with *B. cinerea* were returned to the bags on day 4 and held up to day 14, when the spores were counted.

The spore content of fungus colonies was determined as follows: 20 ml of distilled water were poured into each of the 10 plates per treatment, the colony surface was rubbed gently with a robber policeman, and the spore suspension from all 10 plates was collected in a beaker. The composite spore suspension of the pathogen for each treatment was centrifuged at 16,320 × g for 20 min (Sorvall RG 2-B), and the spore pellet was resuspended in a volume of distilled water determined suitable for counting the spores under a light microscope ( × 100) with a haemacytometer. Twelve spore counts were made per treatment, and the number of spores for every square millimeter of fungus colony was extrapolated. Suppression (percent) of mycelial growth or suppression of sporulation was calculated in the same manner as for disease suppression.

All of the above experiments were performed twice. Since the results of each experiment were similar. the data were averaged and analyzed using SAS (SAS, 1982). The significant F test analysis was followed by the *t* test or Duncan's multiple range test. The data on percentage suppression were transformed into angles before analysis. The Pearson product-moment correlation method was used to determine the correlation coefficients between data on suppression of diseases and those on suppression of mycelial growth and sporulation of pathogens.

Although *M. mucedo* had a narrower host range than *B. cinerea,* it was more virulent on some vegetables in storage (Table 1). *M. mucedo* severely infected cucumber, eggplant, pepper, and tomato fruits, but not bean, carrot, celery, and onion that were stored at 13C for 7 days. Also, it did not attack carrot, celery, onion, or parsnip stored at 1C for 70 days. *B. cinerea* infected all of these crops stored at 1 or 13C.

After 7 days at 13C, however, *M. mucedo* was most pathogenic to eggplant and tomato, while *B. cinerea* was most damaging to celery, cucumber, and eggplant (Table 1). After

Table 3. The suppression of mycelial growth and sporulation of *Mucor mucedo* and *Botrytis cinerea* in eggplant-extract agar in various atmospheres at 13C.

| Atmosphere (%) | | | Suppression (%)[z,y] | | | |
|---|---|---|---|---|---|---|
| | | | Mycelial growth | | Sporulation | |
| $O_2$ | $CO_2$ | CO | *Mucor mucedo* (4 days) | *Botrytis cinerea* (4 days) | *Mucor mucedo* (4 days) | *Botrytis cinerea* (14 days) |
| 21.0 (air control) | | | 0.0 c | 0.0 c | 0.0 e | 0.0 e |
| 1.5 | 0 | 0 | 11.0 b | 2.2 c | 63.2 d | 73.5 cd |
| 1.5 | 4.0 | 0 | 16.3 ab | 12.1 ab | 66.6 d | 90.9 b |
| 1.5 | 0 | 7.5 | 19.4 a | 17.5 a | 80.2 c | 100 a |

[z]Suppression (%)ofthe mycelial growth or sporulation was calculated as follows: colony diameter (cm) or number of spores/$mm^2$ in the control minus colony diameter or number of sporesin the atmospheres divided by the colony diameter or number of spores in the control multiplied by 100%. Mean colony diameters of *M. mucedo* and *B. cinerea* in the control were 7.2 and 4.5 cm, respectively . The numbers of spores/$mm^2$ of *M. mucedo* and *B. cinerea* in the control were 7820 sporangiospores and 2687 conidiospores, respectively.

[y]Means in the mycelial growth or sporulation comparisons followed by the same letter are not significantly different (*t* test, $P = 0.05$).

70 days at 1C, the pathogenicity of *B. cinerea* did not differ on carrot, celery, onion, and parsnip.

At 13C, both *M. mucedo* and *B. cinerea* lesions initially were tan-colored and water-soaked, but, as the lesions progressed, the host tissues softened and collapsed when handled (Fig. 1). Growth of grayish-white mycelium of either pathogen covered the disease lesion. Themycelium of *M. mucedo* covering the lesions developed sporangiophores with blackish sporangia within 7 days after inoculation, while the *B. cinerea* mycelium covering the lesions produced conidiophores with brownish conidia 14 days after inoculation.

The mean diameter of mucor rot lesions (8.5 cm) on eggplant in the air control after 7 days at 13C was larger than that of gray mold lesions (4.2 cm) under similar conditions (see footnote of Table 2), The lesions of both diseases were significantly reduced in CA. However, there was no difference between the suppression of mucor rot under different CA treatments (Table 2). With gray mold, the best suppression was in 7.5% CO + l.5% $O_2$, and the suppression in 4% $CO_2$ + 1.5% $O_2$ was not different from that in 1.5% $O_2$. Within each atmosphere, the suppression of mucor rot was less than that of gray mold.

These results were similar to those reported by others (Adair, 1971; Geeson and Browne, 1980; Reyes and Smith, 1986) that indicate that the amount of suppression of vegetable diseases in CA storage varies with the disease. Adair (1971) reported that the severity of gray mold and watery soft rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) of cabbage were reduced in 1.4% $O_2$ after 6 months at 1C, whereas rot caused by *Fusarium roseum* Lk. was unaffected. Geeson and Browne (1980) found that cabbage could be stored for at least 29 weeks in 5% to 6% $CO_2$ + 3% $O_2$ at 0 to 2C with reduced incidence and severity of gray mold, but rots caused by *Alternaria alternata* (Fr.) Keissler, *A. brassicicola* (Schw.) Wiltshire, and *Mycosphaerella brassicicola* (Duby) Lindau were not consistently controlled by CA treatment. Reyes and Smith (1986) have shown that an 8-week treatment of 7.5% CO + 1.5% $O_2$ at 0 to 1C suppressed gray mold and watery soft rot diseases of celery better than 4% $CO_2$ + 1.5% $O_2$ 0r 1.5% $O_2$, and that 4% $CO_2$ + 1.5% $O_2$ did not significantly affect gray mold. The present results with eggplant indicate that 7.5% CO + 1.5% $O_2$ suppressed gray mold more than either 4% $CO_2$ + 1.5% $O_2$ or 1.5% $O_2$, and that suppression of mucor rot did not differ in these CAs.

The growth (7.2 cm in diameter) of *M. mucedo* on EEA after 4 days in the air control at 13C was greater than that of *B. cinerea* (4.5 cm in diameter) (see footnote of Table 3). The rate of growth for each fungus was suppressed in all atmospheres, except in 1.5% $O_2$, where growth of *B. cinerea* did not significantly decrease (2.2% suppression) (Table 3). The greatest suppression of growth for both organisms was in 7.5% CO + 1.5% $O_2$. The suppression of *M. mucedo* in 4% $CO_2$ + 1.5% $O_2$ was not significantly different from that in 1.5% $O_2$ or 7.5% CO + 1.5% $O_2$. Although CA suppressed growth of these fungi, suppression of *M. mucedo* was not significantly different from that of *B. cinerea* in any CA, except in 1.5% $O_2$, in which *M. mucedo* was suppressed more.

*M. mucedo* sporulated after 4 days (7820 sporangiospores/$mm^2$ of growth) on EEA in the air control at 13C. The amount of sporulation was suppressed in all atmospheres, but the amount of suppression in 1.5% $O_2$ and 4% $CO_2$ + 1.5% $O_2$ did not significantly differ. In contrast, *B. cinerea* did not sporulate until the 14th day (2687 conidiospores/$mm^2$) in the air control, and no sporulation occurred in 7.5% CO + 1.5% $O_2$.

Although measured at different times, the suppression of sporulation of *M. mucedo* in all CAs, except in 1.5% $O_2$, was less than that of *B. cinerea.*

*These* results are consistent with those reported by others (Adair, 1971; Sommer et al., 1981) that indicate that inhibition of growth and sporulation of postharvest pathogens on agar in CA varies with the organism. Adair (1971) has reported that growth of *B. cinerea* and S. *sclerotiorum* on PDA was reduced after 48 hr in 0.8% $O_2$ at 24C, but that growth of *Alternaria tenuis* auct. sensu Wiltshire, *F. roseum,* and *Rhizoctonia solani* Kuhn was not reduced and that no reduction in growth of *F. roseum* was observed in 4.7% $CO_2$ + 0.8% $O_2$. Similarly, Sommer et al. (1981) have shown that the daily growth rate of *Monilinia fructicola* (Wint.) Honey on PDA maintained in 10% CO + 2.2% $O_2$ at 20C was suppressed by ≈ 70%, whereas growth of *B. cinerea* and *Penicillium expansum* Lk. was suppressed by 30% to 40%.

The suppression of mucor rot on eggplant in CA was highly correlated with suppression of growth ($r = 0.78$) and sporulation ($r = 0.71$) of *M. mucedo* on agar. For gray mold, disease suppression was also highly correlated with the suppression of mycelial growth ($r = 0.76$) and sporulation ($r = 0.87$) of *B. cinerea.*

These results further indicate that the effect of CA is largely on the pathogen (or disease), and, therefore, vegetables suspected to be affected by a given pathogen may be stored together for economic reasons. Controlled atmosphere also delays senescence, and the resulting improved physiological condition of the host may enable it to resist decay by some pathogens during storage.

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# Postharvest Handling of *Physostegia purpurea* Cut Flowers

**John W. Kelly**[1]
*Department of Horticulture, Clemson University, Clemson, SC 29634*

**Terri W. Starman**[2]
*Department of Plant and Soil Science, Southern Illinois University, Carbondale, IL 62901*

*Additional index words.* false dragonhead, lion's heart, obedient plant, senescence, storage, ethylene, vase life

***Abstract.* *Physostegia purpurea* Blake is a native, herbaceous perennial that has potential as a field-grown cut flower. *Physostegia* stems were harvested with one third of the florets open and were recut underwater in the laboratory. Fresh cut flowers treated with silver thiosulfate (STS) and held in a 2% preservative solution lasted 14 days, while control stems in deionized water (DI) lasted 6 days. Cut stems placed in darkness at 0C for 1 week had 8 days of vase life after removal from storage and treatment with STS and preservative, while stems held in DI after storage lasted only 4 days. Stems held dry at 22.5C and 43% RH for 8 hours before being placed in preservative had similar vase life as flowers placed in preservative immediately after harvest.**

Field production of cut flowers has evoked renewed interest in recent years. Annual and perennial herbaceous garden plants have received increased emphasis as potential cut flower crops. A major problem encountered in the field production of these crops is that very little cultural or postharvest handling information is available.

*Physostegia purpurea* is a herbaceous perennial that has potential as a cultivated cut flower. The species is adapted to moist, low areas and flowers abundantly in purple spikes during April and May in the southern United States. The flower buds, ≈40 per stem, mature acropetally. The main characteristics that recommend *P. purpurea's* use as a cut flower are an upright growth habit, with an abundance of attractive flowers on long, sturdy stems, and early flowering. These traits may allow producers to take advantage of seasonally higher prices for cut flowers.

If a cut flower is to be successfully marketed, the postharvest handling techniques must be determined, but no information has been published on *P. purpurea.* The objective of this research was to determine the effects of silver thiosulfate (STS), commercial preservative, ethylene, and storage on longevity of *P. purpurea* cut flowers.

*P. purpurea* stems were harvested from field plantings in late April and experiments were repeated in early May. Based on results from preliminary experimentation, all stems were harvested with about one third of the flowers open. Cut flowers were immediately transported to the laboratory and stems were cut under deionized water (DI) to 50 cm. The lower one third of the foliage was removed and plants were handled as described below.

*Vase life determinations.* Cut stems were treated individually in graduated test tubes with either 2 mM STS or DI until each stem had absorbed 1 ml of solution. Silver thiosulfate was prepared as described by Cameron and Reid (1981). Three replications of five samples each from the treatments were placed individually in either 0, 10.5, or 21 g Florever/liter, a preservative solution (Floralife, Chicago), made up with DI water. Cut flowers were evaluated at 22.5 ± 1.5C, 45% ± 10% RH, and 16.7 ± 1.5 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetic photon flux (PPF) from cool-white fluorescent lights with a 24-hr photoperiod. Cut stems were maintained until vase life was considered terminated, which was when one half of the florets on a stem had wilted.

*Ethylene sensitivity.* Ten stems per treatment were cut under water to 15 cm and placed individually into graduated test tubes containing 1 ml of either 2 mM STS or DI. Based on data from a preliminary screening and the preservative study noted above, each flower stem was carefully monitored and allowed to take up 0.3 ml of either STS or DI and then was placed in a 2% preservative solution. Cut stems were held under the environmental conditions described above. At ≈1000 HR each day, stems were weighed and placed in 25-ml Ehrlenmeyer flasks that were mounted inside a 0.94-liter glass jar and contained 20 ml of preservative solution. The lid of the jar was equipped with a rubber serum stopper for gas sampling. Internal atmosphere of the glass jars was flushed with compressed air for 5 sec before sealing. After 2 hr, l-ml gas samples were removed and

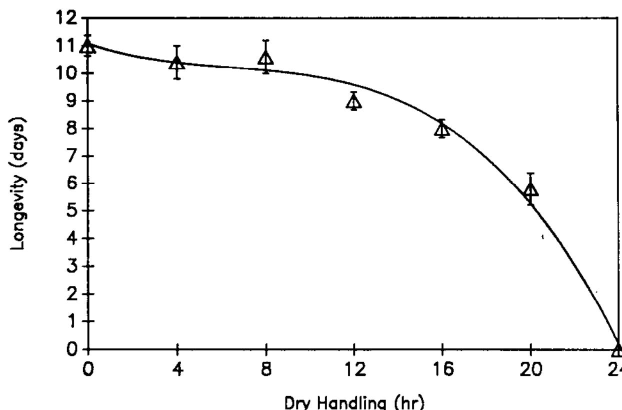


Fig. 1. Longevity of *Physostegia purpurea* as affectcd by duration of dry handling at 22.5C. Bar indicates mean ± SE.

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[1]Associate Professor. To whom reprint requests should be addressed.

[2]Assistant Professor.