Fungicidal Control of Infection by *Penicillium* spp. of Precooled Tulip Bulbs in a Modified Atmosphere Package

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ABSTRACT

Prince, T. A., Herner, R. C., and Stephens, C. T. 1987. Fungicidal control of infection by *Penicillium* spp. of precooled tulip bulbs in a modified atmosphere package. Plant Disease 71:307-311.

Basal plates of precooled tulip bulbs maintained in sealed modified atmosphere (MA) packages of low-density polyethylene film for 3 or 4 wk became infected with species of *Penicillium*. High levels of infection in the basal plates were correlated with increased ethylene levels in the packages and increased floral abortion during subsequent forcing of the bulbs. Prochloraz and etaconazole dip pretreatment of the bulbs and captan dust application before packaging reduced the occurrence of basal plate rot caused by *Penicillium* spp. and allowed excellent root growth and flowering of the bulbs. Benomyl, captan, and chlorine dip pretreatments provided poor control of basal plate infection in the packages.

The design and initial testing of a specialized package for marketing precooled tulip bulbs (*Tulipa gesneriana* L. 'Kees Nelis') without a refrigeration requirement has previously been outlined (14). This system consists of a consumer-size package of five precooled tulip bulbs sealed in low-density polyethylene film (LDF-301, Dow Chemical). The interaction of bulb respiration and differential permeability of the polymeric film to atmospheric gases results in the establishment of modified atmosphere (MA) conditions within the package. Atmospheres of 3–5% O_2 have previously been demonstrated to reduce post-precooling bulb respiration rates, to reduce the detrimental effects of ethylene exposure, and to ultimately increase the time period the bulbs can be held at ambient temperatures before planting (15). Bulbs subjected to normal atmospheric conditions during a holding period yield a high percentage of aborted flowers. Aborted flowers showed arrested development leading to desiccated and faded petals. Although the MA package reduced this abortion in preliminary trials, fungal growth was observed on the bulb basal plates during storage. In addition to being unsightly and rendering the bulbs unacceptable from a consumer viewpoint, the fungus or fungi appeared to cause a basal plate rot that was responsible for floral abortion.

The high relative humidities that develop in some MA packages have been shown to create excellent environments for infection by fungi and bacteria. Disease-causing organisms have been observed to be detrimental when cut tulips (11), carnations and chrysanthemums (7), bell peppers (3), avocados (12), and flowering pot and bedding plants (9) are sealed in various polymeric films. Apparently, the slight growth suppression of disease organisms by atmospheres of 2–3% O_2 and 5% CO_2 observed in vitro (6) is not sufficient to counteract the enhancement of infection by the high humidity levels in sealed packages.

Fungicide or surface-disinfectant pretreatment of commodities has been instrumental in the success of some sealed packages. A chlorine dip treatment, which controlled mold development on tomatoes in polyvinylchloride and polyethylene packages (17), led to a fruit shelf life of 21 days at 25 C. Benomyl and thiabendazole pretreatments controlled occasional infections by *Gloeosporium musarum* (Cke.) Massee of bananas in large, sealed, polyethylene shipping containers (18,19), allowing this MA packaging technique to be used commercially for distant market shipment of bananas (22).

During our studies of bulb packaging, fungi isolated from diseased tulip bulbs were identified as *Penicillium* spp. Detailed studies of the identification, pathogenicity, fungicide resistance, and ethylene-producing ability of these isolates are reported elsewhere (T. A. Prince, C. T. Stephens, and R. C. Herner, *unpublished*). Specifically, three separate isolates of *P. corymbiferum* (Westling) Samson, Stolk, & Hadlok and one isolate of *P. rugulosum* Thom. were found to be pathogenic on bulb basal plates of tulip. One of the *P. corymbiferum* isolates was found to be benomyl-tolerant and to produce ethylene in pure culture.

The control of infection by *Penicillium* spp. of precooled tulip bulbs in a sealed polymeric film package could make the marketing of these precooled bulbs under MA conditions a commercial reality. This study was conducted to determine the effectiveness of various fungicide pretreatments in controlling basal plate infection caused by these pathogenic *Penicillium* isolates on the packaged bulbs. In addition, the impact of these pathogens on ethylene levels in the package and the subsequent rooting and flowering ability of the bulbs was investigated.

MATERIALS AND METHODS

In the autumn of 1981 and 1982, tulip bulbs (12–14 cm in circumference) were shipped to East Lansing, MI, from the Netherlands in open tray cases. The shipping/arrival dates were 11 September/6 October 1981 and 16/30 August 1982. Temperatures were 13–17 C during the first shipment period and 10 days at 17–20 C followed by 4 days at 7–15 C during the second shipment period. All bulbs had reached stage G (trilobed gynoecium formed) upon arrival and were stored at 13 C (1981) or 17–20 C (1982) until the 5 C precooling period began.

Year 1 (1981). Kees Nelis bulbs were precooled in open tray cases at 5 C and 80–90% relative humidity (RH) from 8

Michigan Agricultural Experiment Station Article 11499.

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Accepted for publication 29 July 1986.

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October to 30 December (12 wk). At the end of the precooling period, the bulbs were treated with various fungicides before packaging in LDF-301 film. Bulbs used in this experiment showed no visual symptoms of disease but were assumed to have some level of natural inoculum. Fungicides tested were benomyl (Benlate 50WP), etaconazole (20) (Vangard 10WG), prochloraz (21) (BTS-40-542 40EC), and captan (Captan 50WP). All fungicides were applied by dipping the bulbs for 20 min in well-agitated tap water suspensions in 4-L containers at 21 C. Two rates of each fungicide were used (Table 1). Captan also was applied as a dust. The low rate of captan dust was prepared from Captan 50WP and talc in a ratio of 1:4. Bulbs dipped in water without fungicide and nondipped bulbs were used as controls. All dipped bulbs were thoroughly dried under flowing air from a small fan before packaging.

Table 1. Fungicidal control of infection by *Penicillium* spp. of bulb basal plates during 3 wk of storage at 20 C in LDF-301 film packages and subsequent flowering of these bulbs (year 1)

Prepackaging treatment	Rate (μg a.i./ml)	Infected bulbs[x] (no.)	Infection per plate (%)	Normal flowers[y] (%)	Root fr wt[z] (g)
H_2O dip	···	5.0 a	64 b	15 c	0.4 fg
No dip	···	1.7 b	24 c	87 a	3.3 bc
Benomyl	1,000	5.0 a	88 a	5 c	0.1 g
	2,000	5.0 a	86 a	5 c	0.1 g
Prochloraz	300	0.5 c	7 c	95 a	3.8 ab
	600	1.0 bc	5 c	95 a	3.8 ab
Etaconazole	120	0.5 c	5 c	100 a	5.0 a
	240	0.5 c	12 c	100 a	4.2 ab
Captan	1,200	4.5 a	54 b	50 b	1.5 de
	2,400	4.5 a	52 b	45 b	1.4 e
Captan dust					
10%	···	1.2 bc	20 c	90 a	4.4 ab
50%	···	1.0 bc	13 c	95 a	4.6 ab
Nonpackaged					
H_2O dip	···	1.7 b	6 c	40 b	0.9 ef
No dip	···	0.5 c	7 c	60 b	2.3 cd

[x]Average of four replicates of five bulbs. Mean separation within columns by Waller-Duncan multiple comparisons procedure at $P = 0.05$.
[y]Nonstored control bulbs yielded 100% normal flowers.
[z]Root fresh weight data analyzed on log $(x + 1)$ transformed scale.

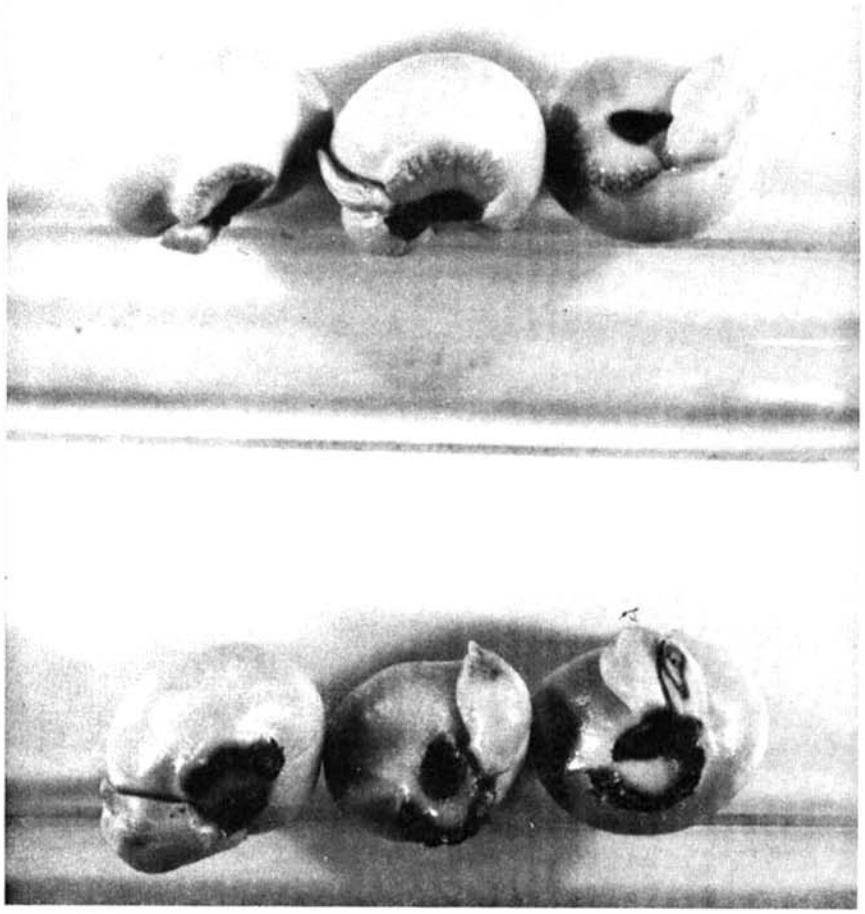

Fig. 1. Infection by *Penicillium* spp. of precooled Kees Nelis tulip bulb basal plates pretreated with **(top)** etaconazole at 120 μg a.i./ml and **(bottom)** benomyl at 2,000 μg a.i./ml and packaged in LDF-301 film for 3 wk at 20 C (year 1).

Five bulbs were heat-sealed inside each MA package constructed of LDF-301 low-density polyethylene film. Four replicate packages containing bulbs from each fungicide or control pretreatment were placed randomly in a room at 20 C (40–50% RH) for 3 wk of storage. Four replicates of nonpackaged, water-dipped, and nondipped bulbs were also stored as controls. The package was composed of 0.08 M^2 of film surface area (20 × 20 cm, top and bottom) with 500 ml of package headspace. Two lengths (about 3 cm) of adhesive tape (Scotch Patch and Repair Tape) were used as gas sampling ports on the film surface of the packages. The top length of tape was used to seal previous sampling holes on the bottom length of tape. The CO_2, O_2, and ethylene levels in the packages were monitored at various intervals during storage by withdrawing a 2-ml (CO_2 and O_2) or 1-ml (ethylene) sample through the sampling ports for gas chromatographic analysis.

At the end of the storage period, the bulbs were removed from the packages and the bulb tunics were peeled from the bulbs. In addition to being a standard procedure of forcing precooled bulbs, removal of the tunic allowed for evaluation of infection by *Penicillium* spp. The number of bulbs with infected basal plates in each package was determined by visual inspection. For each infected bulb, the percentage of the basal plate damaged by *Penicillium* surface growth was estimated to the nearest 5% as an indication of disease severity.

After inspection, the five bulbs in each package were planted in a mix of 20% vermiculite, 20% perlite, and 60% peat in a 15-cm pot. After planting, each pot was drenched with a 0.2% benomyl and 0.4% ethazol solution as a standard control of *Pythium* and *Rhizoctonia*. The pots were placed randomly on a greenhouse bench under a minimum night temperature of 16 C and grown under normal cultural procedures. Upon flowering (about 20 days after planting), the percentage of normal flowers obtained in each pot was determined. The bulbs were subsequently removed from the pots and the soil was carefully washed from the roots. The roots were blotted dry and cut from the bulbs for fresh weight determination.

Year 2 (1982). Kees Nelis bulbs were precooled at 5 C and 80–90% RH from 17 September to 10 December (12 wk).

Some *Penicillium* growth was observed on the bulb tunics during the precooling period. To avoid disease development during precooling, all bulbs were dusted with talc on 23 November to lower the available surface moisture for pathogen growth while avoiding fungicide use that would confound later studies. At the end of the precooling period, the bulbs were inoculated by dipping the bases of the bulbs in a *Penicillium* spore suspension (about 10^7/ml) for a few seconds. Although bulbs chosen for this experiment appeared visually clean, no attempt was made to remove naturally occurring inoculum before inoculation. The spore suspension was prepared by mixing three pathogenic isolates of *P. corymbiferum* and one isolate of *P. rugulosum* that were isolated from diseased bulbs received in the first year of this experiment. One of the *P. corymbiferum* isolates used was shown to be an ethylene producer and benomyl-tolerant (T. A. Prince, C. T. Stephens, and R. C. Herner, *unpublished*). A few drops of Tween 20 surfactant were added to the suspension to facilitate bulb wetting. After inoculation, the bulbs were allowed to dry before further treatment.

Etaconazole (240 μg a.i./ml), prochloraz (600 μg a.i./ml), or bleach (6,000 ppm available chlorine, pH 7.6) pretreatments were applied before bulb packaging. In addition, water-dipped and nondipped control bulbs were included. The bulbs were pretreated and packaged as in year 1 and stored for 4 wk at 20 C. Percent basal plate infection and flower abortion were evaluated as in year 1, but root fresh weight was not determined.

RESULTS

Year 1. All packages contained 4.5–5.5% O_2 and 4.0–5.0% CO_2 by the 11th day after package sealing, whereas package ethylene levels at the end of storage ranged from about 0.1 to 0.9 μl/L. Ambient levels during storage were about 0.10% CO_2, 20.2% O_2, and 0.05 μl/L of ethylene.

Fungicidal control of infection by *Penicillium* spp. of the bulb basal plates during storage is shown in Table 1. Infection was observed as growth of mycelia resulting in a brown discoloration and rotting of the underlying tissue. Dipping the bulbs in tap water before packaging resulted in a significantly greater number of infected bulbs in a package and a significantly greater infected surface area on the basal plate when compared with nondipped, packaged control bulbs. All bulbs pretreated with benomyl before packaging became infected, with nearly the entire basal plate of each bulb colonized by *Penicillium* spp. (Table 1, Fig. 1). The number of captan-dipped bulbs affected by *Penicillium* was not significantly different from the benomyl-treated or the water controls, although the percentage of disease development on each basal plate was statistically less in the benomyl-treated bulbs. Etaconazole and prochloraz pretreatment resulted in a significantly lower number of infected bulbs in a package and reduced colonization by *Penicillium* spp. of the basal plates compared with water-dipped bulbs. Prochloraz at 300 μg a.i./ml and etaconazole at either rate also resulted in fewer infected bulbs in a package than nondipped controls, although the extent of colonization by *Penicillium* spp. on a once-infected individual plate was not statistically different from nondipped controls. Disease severity on bulbs dusted with captan before packaging was not statistically different from that on prochloraz- or etaconazole-dipped bulbs or from the nondipped bulbs. There were some infected bulbs in nonpackaged treatments; the water dip significantly increased the number of infected bulbs per replicate compared with the no-dip treatment. No evidence of phytotoxicity to the bulbs upon removal from the packages was visible after any of the fungicide treatments.

Fungicidal pretreatment effects on subsequent root growth and flowering of the bulbs are shown in Table 1. Water or benomyl dips resulted in extremely poor root growth and flowering of the bulbs after 3 wk in the MA packages. Flowers with dried and papery petals were rated abnormal. The captan drench pretreatment also reduced normal flowering and root growth, although not as severely as the water or benomyl dip pretreatment. Vigorous root growth and a high number of normal flowers were obtained from the nondipped, etaconazole- or prochloraz-dipped, or captan-dusted treatments. Nonpackaged bulbs showed poor root growth and flowered poorly.

Year 2. Nearly all of the nondipped, water-dipped, or chlorine-pretreated bulbs in each package became infected during 4 wk of storage (Table 2). The percentage of infection per basal plate was greatest in the chlorine pretreatment (56%). The number of infected bulbs in each package after etaconazole or prochloraz pretreatments was significantly lower than in the water, chlorine, or no-dip pretreatment. In addition, there was significantly less infection per basal plate in the etaconazole and prochloraz pretreatments than in the chlorine pretreatment. Half of the nonpackaged bulbs became infected, although the percentage of infection on the individual plates was an average of 10%.

Flowering of the bulbs after storage is documented in Table 2. Nonpackaged bulbs developed only abnormal flowers upon forcing in the greenhouse. All packaged bulbs yielded a significantly greater percentage of normal flowers than the nonpackaged bulbs. Of all packaged bulbs, the chlorine-treated bulbs produced the lowest percentage of normal flowers (45%). The nondipped, etaconazole-, or prochloraz-pretreated bulbs yielded 90% or more normal flowers upon forcing.

Package ethylene levels during the 4 wk of storage are shown in Table 3. Ethylene accumulation above ambient levels was measured in all packages. By 27 days of storage, the packages containing water- or chlorine-dipped and nondipped bulbs had significantly greater ethylene levels than the packages of etaconazole- or prochloraz-pretreated bulbs. Package CO_2 and O_2 levels were similar to those during year 1.

DISCUSSION

Infection of bulb basal plates by *Penicillium* spp. during storage decreased the effectiveness of the MA packages in maintaining flowering ability of precooled tulip bulbs. In addition to the cosmetic damage to the bulbs, the disease appeared to reduce rooting and to lead to higher levels of floral abortion upon forcing of the bulbs. The most direct effect was damage to the basal plate with its slightly emerged root initials. During isolation and washing of the roots to determine fresh weight, lack of root growth from diseased portions of the

Table 2. Fungicidal control of infection by *Penicillium* spp. of bulb basal plates inoculated with a mixed spore suspension[x] and stored for 4 wk at 20 C in LDF-301 packages and subsequent flowering of these bulbs (year 2)

Prepackaging treatment	Infected bulbs[y] (no.)	Infection per plate (%)	Normal flowers[z] (%)
H_2O	4.7 a	35 ab	70 bc
No dip	4.2 a	20 b	90 ab
Chlorine (6,000 ppm)	4.7 a	56 a	45 c
Etaconazole (240 μg a.i./ml)	0.5 c	12 b	95 ab
Prochloraz (600 μg a.i./ml)	0.7 c	11 b	100 a
Nonpackaged	2.5 b	10 b	0 d

[x] Bulb basal plates inoculated with mixed spore suspension of isolates of *P. corymbiferum* and *P. rugulosum*.

[y] Average number per package of five bulbs. Mean separation within columns by Duncan-Waller multiple comparisons procedure at $P = 0.05$.

[z] Nonstored control bulbs yielded 100% normal flowers.

basal plates was commonly observed. The treatments that resulted in 50% or greater disease of the basal plate surface led to the poorest root growth (Table 1). This poor root growth probably led to some of the floral abortion observed after these treatments.

In addition to direct damage to the basal plates by the disease, the ethylene accumulation in the packages containing diseased bulbs may have been partially responsible for the floral abortion and reduced root growth shown in Table 3 (5). Several factors could have contributed to the ethylene accumulation in the packages. A portion of this ethylene was probably produced by the bulbs as a stress response (1). Healthy bulbs also have been shown to produce increased amounts of ethylene as the length of the precooling period is increased (16). This may account for some of the ethylene accumulated in packages of bulbs with few disease symptoms. In addition to ethylene production by the bulbs, the fungi may have produced ethylene during infection. One of the isolates of *P. corymbiferum* obtained from diseased bulbs and used for inoculation has been shown to produce ethylene in pure culture (14).

Abnormal flowering (dried and papery petals) and reduced root growth observed in these studies have been previously shown to result from exposure of tulip bulbs to as little as 0.3 μl/L of ethylene (5). However, previous studies with precooled bulbs revealed substantial reduction of the detrimental effects of exposure to 5 μl/L of ethylene if the bulbs were held under a 3–5% O_2 atmosphere in a constantly ventilated system. A similar protective effect should have resulted in the low-O_2 atmosphere of the MA packages where ethylene levels were less than 5 μl/L even when disease and fungi were present. However, under the static conditions inside the MA package, the ethylene levels in the microenvironment surrounding the bulbs may have been much higher than package levels indicated. Additionally, the MA packaging system required about 10 days to reach the desired low-O_2 conditions. This delay may have allowed some ethylene damage to occur before package equilibrium.

Only moderate levels of basal plate infection by *Penicillium* spp. were observed on nondipped, packaged bulbs in these studies (Tables 1 and 2). This suggests that fungicide pretreatment may not always be necessary for the bulbs to be marketed successfully in the MA package. However, three factors may impact fungicide application necessity: 1) effects of temperature fluctuation in the marketplace on the package environment, 2) possible differences in bulb susceptibility from year to year, and 3) bulb condition.

The packaging trials reported here were conducted under a constant 20 C. Under normal marketing conditions, temperature fluctuations between 15 and 25 C are expected and can be tolerated by the package with only minor changes in the gaseous atmosphere (14). However, a decline in temperature results in condensation of water on the inner package surface resulting from the near-saturated relative humidity in the package headspace. This would probably enhance disease development by providing a more ideal environment for spore germination and hyphal penetration. Both free water and nutrients leached from host tissue have been shown to be necessary for germination of *P. digitatum*, Sacc. spores (10,13).

Bulb susceptibility to infection may vary from year to year. A documented example of this is the difference in susceptibility of tulip bulbs to infection by *Fusarium oxysporum* (Schlecht.) emend. Snyd. & Hans. f. sp. *tulipae* Apt. that has been linked to the earliness of bulb harvest in the Netherlands (8). Dipping the bulbs in water as a control significantly increased the number of infected bulbs and growth of the fungus on the basal plates compared with nondipped control bulbs in year 1 (Table 1). This was probably due to spread of inoculum from the surface of the bulb tunics to the underlying basal plates during the 20-min dipping period. Some growth of *Penicillium* is commonly seen on the tunic surface upon arrival of bulbs from the Netherlands. Under normal bulb-forcing techniques, it causes minimal problems (4). During harvesting, handling, and shipping, cracks sometimes develop in the normally continuous tunic near the area of the basal plate. These openings may allow passage of spores from the tunic surface to the basal plate, particularly in the presence of water. Rough handling of bulbs may lead to more tunic cracking and ultimately more diseased basal plates in this new packaging system. For all of the above reasons, fungicide application before packaging appears advisable to prevent disease occurrence.

The response to fungicide or disinfectant pretreatment ranged from excellent (etaconazole and prochloraz) to extremely poor (benomyl) (Table 1). Etaconazole and one rate of prochloraz significantly reduced the number of infected bulbs even over the no-dip treatment in the year 1 study. In the year 2 inoculation studies, an average of 0.6 bulb per package was infected in the etaconazole and prochloraz treatments compared with 4.2 bulbs in the next best treatment, the no-dip controls. Captan pretreatment was ineffective as a dip application but appeared to control infection when applied as a dust. However, the dust was unsightly upon removal of the bulbs from the packages and would probably be undesirable to the consumer.

The captan dust may have been more effective because it was applied dry, so it was comparable to the no-dip treatment. The drying effect of the talc itself may also have reduced infection. The use of desiccants in the package to reduce infection deserves further investigation.

Neither chlorine nor benomyl dips controlled disease development to any extent. The chlorine apparently did not penetrate the basal plates deeply enough to destroy the inoculum. The benomyl dip did not control the disease compared with water-dipped controls. The ineffectiveness of benomyl is probably due to the presence of the benomyl-tolerant isolate of *P. corymbiferum* used in the inoculation study and previously isolated from diseased bulbs (6).

The success of the prochloraz and etaconazole fungicides may be due to their activity against a benomyl-tolerant isolate of *P. corymbiferum*. Etaconazole appeared to be active at a lower rate than prochloraz. Both of these compounds, at the rates used here, have previously been shown to be active against benomyl-tolerant *P. expansum* isolates on stored apples (2). This study suggests that either of these compounds will be effective in controlling disease caused by *Penicillium* spp. on precooled tulip bulbs in an MA package. However, neither of these compounds has been registered for commercial use.

Table 3. Ethylene levels in LDF-301 film packages of Kees Nelis tulip bulbs after inoculation with a mixed spore suspension of *Penicillium* spp. and subsequent prepackaging fungicide or disinfectant treatment (year 2)[x]

Prepackaging treatment	Ethylene levels (nl/L)[y,z]		
	Day 6	Day 18	Day 27
H_2O dip	39 a	245 a	192 a
No dip	48 a	162 a	278 a
Chlorine (6,000 ppm)	28 a	107 ab	381 a
Etaconazole (240 μg a.i./ml)	27 a	38 c	46 b
Prochloraz (600 μg a.i./ml)	25 a	53 bc	71 b

[x] Bulb basal plates were inoculated with a mixed spore suspension of isolates of *P. rugulosum* and *P. corymbiferum*.

[y] Mean separation within columns by Duncan-Waller multiple comparisons procedure at $P = 0.05$. Data were analyzed on log transformed scale.

[z] External atmospheric ethylene levels during storage were 10–20 nl/L.

ACKNOWLEDGMENTS

We express appreciation to the Holland Bulb Exporters Group, New York, and the International Flower-Bulb Center, Hillegom, the Netherlands, for partial financial support of this research. We also acknowledge Dow Chemical for supplying polymeric films and Mollie Stark and Denise Cerny for technical assistance.

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