Ecology and Epidemiology

Pathogenicity, Fungicide Resistance, and Ethylene Production of *Penicillium* spp. Isolated from Tulip Bulbs

T. A. Prince, C. T. Stephens, and R. C. Herner

Former graduate research assistant, Department of Horticulture, Michigan State University; associate professor, Department of Botany and Plant Pathology; and professor, Department of Horticulture, Michigan State University, East Lansing 48824. Present address of first author: Department of Horticulture, The Ohio State University, 2001 Fyffe Court, Columbus 43210.

Michigan Agriculture Experiment Station Journal Article 12095. Work supported in part by the Dutch Bulb Exporters Association and the International Flower-Bulb Center, Hillegom, the Netherlands.

The technical assistance of T. S. Stebbins is gratefully acknowledged. We also acknowledge Philip B. Mislivic, Ph.D., of the Division of Microbiology, Bureau of Foods, Food and Drug Administration, Washington, DC, for isolate identification.

Accepted for publication 2 December 1987 (submitted for electronic processing).

ABSTRACT

Prince, T. A., Stephens, C. T., and Herner, R. C. 1988. Pathogenicity, fungicide resistance, and ethylene production of *Penicillium* spp. isolated from tulip bulbs. Phytopathology 78:682-686.

Three isolates of *Penicillium corymbiferum* and one isolate of *P. rugulosum* obtained from tulip bulbs (*Tulipa gesneriana* L.) shipped from the Netherlands were shown to be pathogenic on excised basal plates of precooled tulip bulbs. One isolate of *P. corymbiferum* was benomyl resistant when cultured on benomyl amended potato-dextrose agar (PDA). Etaconazole and prochloraz fungicides controlled growth of this isolate in vitro. The benomyl-resistant isolate produced 1.5 μl/cm^2/hr of ethylene when grown on PDA. The other isolates did not produce ethylene on this medium. Inoculation of benomyl pretreated, excised basal plates with a mixed spore suspension of the same two isolates yielded a predominance of benomyl-resistant colonies after 1 wk. Etaconazole pretreatment controlled infection of excised basal plates by both isolates. Ethylene levels in sealed modified atmosphere film packages of bulbs remained similar through 2 wk after bulb basal plates were inoculated with an ethylene producing (benomyl-resistant) or a non-ethylene producing (benomyl-susceptible) isolate of *P. corymbiferum*. However, after 3 wk of storage there were higher ethylene levels in the packages containing the ethylene producing isolate even though the disease levels were similar.

The development of a modified atmosphere package for marketing of precooled tulip bulbs under nonrefrigerated conditions has recently been reported (15). This system is advantageous because it significantly increases shelf-life of precooled bulbs due to development of package atmospheres of 3–5% O_2 and CO_2 and near saturated relative humidity conditions during storage at 20 C. Although this environment is ideal for maintenance of the floral shoot within the bulbs, it also provides an ideal environment for infection of the bulb basal plates by *Penicillium* spp. The first symptom of infection is a brown discoloration of the root initials followed by growth of the fungus and rotting of the tissue (16). Reduced root growth and a higher level of floral abortion occurs in infected bulbs that are forced.

The control of basal plate infection by *Penicillium* spp. appears then to be a limiting factor in the success of this packaging system. During experimental attempts to control infection, bulb pretreatment with benomyl or captan dips were unsuccessful, while pretreatment with two experimental fungicides (17,18) controlled infection (16). Currently, bulbs are being treated in the Netherlands with benlate prior to shipping to control infection by *Fusarium oxysporum* (Shlecht.) emend. Snyder & Hansen f. sp. *tulipae* Apt.

Ethylene production by either the infected bulb tissue or the infecting fungi themselves, or both, may have led to the observed package ethylene accumulations (16). Although ethylene production in vitro by *P. digitatum* Sacc. has been investigated thoroughly (5,6,8,13,21), ethylene production by *Penicillium* spp.

pathogenic to tulip bulbs has not been investigated.

The purpose of this study was to identify and determine the pathogenicity and relative virulence of various isolates of *Penicillium* spp. obtained from tulip bulbs; to evaluate the fungicidal resistance of these isolates; to study competition of a benomyl-resistant and benomyl-sensitive isolate on excised, fungicide pretreated basal plates; and to elucidate the immediate source of ethylene accumulations in packages of infected bulbs.

MATERIALS AND METHODS

Tulip bulbs (12–14 cm in circumference) were shipped to East Lansing, MI, from the Netherlands in open tray cases during the 1981–1982 and 1982–1983 bulb forcing seasons. Temperatures during shipment were 13–17 C (1981–1982) and 17–20 C for 10 days, followed by 4 days at 7–15 C (1982–1983). The shipping/arrival dates were 11 September/6 October 1981 and 16/30 August 1982. The floral shoot in all bulbs had formed a tri-lobed gynoecium before arrival. The bulbs were stored at 13 C (1981–1982) or 17–20 C before precooling. All studies except the isolate competition study were repeated.

Isolation and identification. The isolates of *Penicillium* used for preparation of spore suspensions were cultured from bulbs shipped from the Netherlands on 11 September 1981. Infected bulbs were surface sterilized with a 0.53% sodium hypochlorite solution. Samples of diseased tissue were dissected and placed on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI) under aseptic conditions at 23–26 C. Cultures were resampled and cultured until they appeared pure by visual characteristics. The separated isolates were subsequently maintained by repeated culturing and periodic storage at 5 C. Sample cultures of seven isolates were shipped to Philip B. Mislivic, Division of Microbiology. Bureau of Foods, Food and Drug Administration, Washington, DC, for identification.

Isolate pathogenicity and virulence. Tulip bulbs cultivar Kees Nelis were stored at 5 C and 80–90% RH from 21 December 1981 to 8 June 1982 (24 wk). Bulbs were removed from storage and the tunics (the outer papery covering) were removed to expose the basal plates. Only basal plates visually free of infection by *Penicillium* spp. were selected and cut from the bulbs at the point where the basal plate protrudes from the scale tissue. The basal plates were soaked under gentle agitation for 20 min in 0.53% sodium hypochlorite containing a few drops of Tween 20 surfactant, to reduce any naturally occurring inoculum. After soaking, each basal plate was placed cut surface down on water agar (Difco Laboratories, Detroit, MI) in a 100- × 15-mm petri dish under aseptic conditions. The dishes were placed at 23–26 C for 2 days at which time roots about 5 mm in length had emerged from the basal plates. Each basal plate was then inoculated with a 25-μl droplet of spore suspension or with a sterile water control. All spore suspensions or control solutions were prepared with sterile water with a few drops of Tween 20 surfactant to aid in spore distribution. Spore concentrations were determined with a hemacytometer. Ten basal plates, each in an individual dish, were used for each isolate or water control. The petri dishes were randomly placed on a tray at 23–26 C. Each of the 10 basal plates from each treatment was rated for symptom development after 5, 8, and 11 days. The rating scale was: 1, symptomless; 2, brown discoloration on localized area of suspension application; 3, fungal growth and underlying tissue decay of less than one fourth of discolored area; and 4, fungal growth and tissue decay of greater than one-fourth of discolored area.

Fungicide resistance. Fungicides tested were: benomyl (Benlate 50WP) [methyl-1-(butylcarbamoyl) benzimidazole- 2 yl carbamate], duPont de Nemours & Co., Wilmington, DL; etaconazole (CGA-64251 10WP) [1-[2-(2,4- dichlorophenyl) -4-ethyl, 1,3-dioxolan-2-yl]methyl]-1 H-1,2,4-triazole], Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC; prochloraz (BTS-40-542 40 EC) [1-(N-propyl-N- (2-(2,4,6-trichlorophenoxy) ethyl) carbamoyl) imidazole], The Boots Company Ltd., Wuthingham, England; and captan 50WP [N-trichloromethyl-mercapto-4-cyclohexene- 1,2-dicarboximide], Uniroyal Chemical, Navaticle, CT. Petri dishes (100 × 15 mm) containing PDA with one of each of the above fungicides at different concentrations were prepared (19). Fungicide concentrations were selected to correspond to rates used in actual bulb storage trials (16). Spore suspensions (10^3–10^4 spores per microliter) were prepared from 13-day-old cultures of each isolate. Four drops (40 μl/drop) of each suspension were applied to each prepared dish of PDA. Two petri dishes containing four drops were used for each fungicide/isolate combination. The dishes were placed at 23–26 C for 4 days. They were subsequently rated for fungal growth according to a previously reported (19) scheme: (++), uninhibited growth equal to control plates; (+), growth limited to area of suspension application; and (0), no growth. Before conducting any experiments the second year, the fungicide resistance of the isolates was again determined to elucidate any possible mutations from the repeated culturing.

Pathogen competition. This study was designed to examine the competition of the benomyl-sensitive and resistant isolates on bulb basal plates with or without fungicide pretreatment. Kees Nelis bulbs were stored at 5 C and 80–90% RH from 17 November 1982 until 9 February 1983 (12 wk). Upon removal from storage, basal plates free of visible infection by *Penicillium* spp. were selected and cut from the bulbs. They were dipped in 0.53% sodium hypochlorite for 20 min as described above and subsequently were rinsed twice in sterile water. The basal plates then were dipped for 20 min in either benomyl (2,000 μg a.i./ml), etaconazole (240 μg a.i./ml), or sterile water. Three basal plates then were placed cut surface down under aseptic conditions on 100- × 15-mm petri dishes containing water agar and were held for 48 hr at room temperature (23–26 C).

Two spore suspensions were prepared from 4-wk-old cultures of benomyl-sensitive and -resistant isolates grown at room temperature under 540–1,100 lx from cool-white fluorescent bulbs. The suspensions were adjusted to equal spore concentrations by repeated sampling and counting with a hemacytometer and dilution of the most concentrated suspension. The final adjusted suspensions were sampled 25 times for determination of spore count averages and standard deviations. A third suspension of equal total spore concentration was prepared by mixing equal volumes of the benomyl-sensitive and benomyl-resistant spore suspensions.

Each of the three suspensions or sterile water was applied as three 25-μl drops spaced equidistantly on the basal plate surface. All inoculated basal plates were placed back in the environment described above. The experiment was conducted as a completely randomized 3 × 4 factorial design (three pretreatments × four inoculations including water) with three petri dishes of each treatment/inoculation combination, each containing three basal plates.

After 1 wk, each of the three spots of application on each basal plate was rated with the infection scale described above. An average rating for the nine inoculated areas on the basal plates in each replicate petri dish was then calculated.

Pathogen reisolation was performed to determine the relative extent of infection due to each isolate. The inoculated areas (whether diseased or not) were cut from the basal plates, pooled, and placed in 10 ml of sterile water with one drop of Tween 20 surfactant and subsequently vortexed at high speed for 10 sec. A 0.1-ml sample of the suspension was used to prepare a 10^0, 10^{-1}, and 10^{-2} dilution series. A 0.1-ml sample of each dilution then was spread on PDA in 100 × 15-mm petri dishes. After 3 days at room temperature, the dilution series with the greatest number of distinguishable colonies from each replicate was counted. The isolates were identified by previously observed color differences in the colonies seen on PDA. The top side of the benomyl-resistant colonies had a central area colored blue with a white perimeter. The top side of the nonresistant isolate colonies had a central area colored white with a green perimeter while the bottom side appeared yellow. The ratio of resistant to sensitive colonies as well as number of colonies reisolated per basal plate were calculated.

In vitro isolate ethylene production. Seven-day-old cultures (3–5 cm in diameter) of the isolates of *Penicillium* on PDA in 35- ×

10-mm petri dishes were placed in sterile 473-ml jars. The top half of each petri dish was removed, and the jars were sealed and placed at 20 C for 10 days. Dishes with PDA only were sealed into jars as controls. Each isolate or control was replicated three times. The jar lids were equipped with rubber gas sampling ports that allowed ethylene determination after 3, 6, and 9 days. On day 10, the CO_2 and O_2 levels in the jars also were determined. Gas analysis was performed with a Carle GC-8700 (CO_2 and O_2) and a Varian 1700 (ethylene) gas chromatograph equipped with thermal conductivity and flame ionization detectors respectively.

Ethylene production within packages of precooled bulbs. Kees Nelis bulbs were precooled at 5 C and 80–90% RH from 29 October 1982 until 20 January 1983 (12 wk). At the end of precooling, the tunics were removed and bulbs with basal plates free of visible infection by *Penicillium* spp. were selected. The bulbs were dipped in 0.53% sodium hypochlorite for 20 min and then rinsed in sterile water. The bases of the bulbs were subsequently dipped for 10 min in sterile water or a spore suspension of the ethylene producing (benomyl-resistant) isolate containing 1.8 ($\pm$0.7) $\times$ 10^3 spores per microliter or of the non-ethylene producing (benomyl-susceptible) isolate containing 1.4 ($\pm$0.5) $\times$ 10^3 spores per microliter. The suspensions were prepared as described above. After dipping, the bulbs were dried. Five dip-treated bulbs were heat-sealed in low density polyethylene film (LDF-301, Dow Chemical) packages. The packages were composed of about 0.08 m^2 of film surface area (20 $\times$ 20 cm, top and bottom) with 500 ml of package headspace. Four replicate packages for each treatment were stored for 3 wk at 20 C and 40–50% relative humidity. Two 3-cm lengths of adhesive tape were used as gas sampling ports on the film surface of the packages. The top length of tape was used to seal the previous sampling holes on the bottom length. Gas analysis was performed as described above.

Statistical analysis. Analysis of variance was performed where possible. When the data did not meet the assumptions of the analysis of variance, data transformation was performed before analysis. When transformation was unsuccessful, the Kruskal-Wallis nonparametric statistic was used for testing overall treatment significance, while the Mann-Whitney statistic was used for more specific mean separation (14). These tests are based on ranks and have fewer data assumptions than the analysis of variance.

RESULTS

Isolate identification. Of the seven isolates, three were identified as *P. corymbiferum* (Westling) Samson, Stolk et. Hadlok and one as *P. rugulosum* Thom. Two of the cultures were found to be mixed isolates of *Penicillium*. Another isolate was identified as *Trichoderma lignorum* (Tode) Harz.

Isolate pathogenicity and virulence. The three original *P. corymbiferum*, *P. rugulosum*, and *Trichoderma lignorum* were all found to be pathogenic on tulip bulb basal plates by 11 days (Table 1). The first symptom of infection on the basal plates was a brown discoloration. This was followed by the development of hyphae and visual appearance of conidia and conidiophores, along with decay of the underlying tissue. Isolates 2 and 3 of *P. corymbiferum* and *P. rugulosum* were the most virulent, whereas isolate 1 of *P. corymbiferum* and *T. lignorum* were less virulent. Only the isolates of *Penicillium* were used in further studies.

Fungicide resistance. Growth studies of the four isolates of *Penicillium* spp. on PDA containing four different fungicides indicated that isolate 1 (*P. corymbiferum*) was highly benomyl resistant (Table 2). Even at the high rate of 2,000 μg/ml, the growth of the fungus was similar to that seen on control plates. Isolate 1 did not grow on PDA containing etaconazole or prochloraz fungicides.

A slight growth of isolates 1, 2, and 4 was observed on PDA containing captan. This growth was limited to the area on the agar where the suspension was applied. Growth of isolates 2 and 4 was prevented by all other fungicides tested, while the growth of isolate 3 was prevented by all fungicides including captan. Similar results were obtained when these four isolates were retested for fungicide sensitivity at the beginning of the 1982–1983 bulb forcing season.

Pathogen competition. Suspension spore counts were statistically equal with 1.7 ($\pm$0.9) $\times$ 10^4 spores per microliter for the benomyl-resistant (ethylene producing) isolate and 1.6 ($\pm$0.8) $\times$ 10^4 spores per microliter for the benomyl-sensitive (nonethylene producing) isolate. The results of the pathogen competition studies are shown in Table 3. For pathogen reisolation, the 10^{-1} or 10^2 dilution was counted for the benomyl and water control pretreatments, while the 10^{-1} dilution exclusively was counted for the etaconazole pretreatment. A small number of unidentifiable colonies appeared in the more concentrated dilutions from the water pretreated, water inoculated basal plates. These were not included in the total count of sensitive and resistant colonies.

Inoculation of benomyl pretreated excised basal plates with the resistant isolate of *P. corymbiferum* or the isolate mixture led to a significantly greater average infection rating than that observed on water inoculated basal plates after 1 wk, although the average rating of the sensitive isolate was not significantly different from the other inoculations. Basal plate browning was the only symptom of infection caused by the sensitive isolate on the benomyl pretreated basal plates while there was fungal sporulation and hyphal growth of the resistant isolate. The number of colonies obtained from spores reisolated from the benomyl pretreated basal plates that were inoculated with the sensitive isolate were significantly less than from inoculation with the resistant or the mixed isolates. The observed ratio of resistant to sensitive colonies obtained after spore reisolation from benomyl pretreated basal

TABLE 1. Pathogenicity and virulence of *Penicillium* spp. and *Trichoderma lignorum* on excised root plates of precooled tulip bulbs[x]

Isolate	Species	Infection rating[y] Day 5	Day 8	Day 11
1	*P. corymbiferum*	1.8 a	2.5 ab	2.8 b
2	*P. corymbiferum*	3.5 c	3.9 c	3.9 c
3	*P. corymbiferum*	3.5 c	3.5 c	3.6 c
4	*P. rugulosum*	3.1 bc	3.4 bc	3.7 c
5	*T. lignorum*	2.7 b	2.4 a	2.5 b
Control[z]		1.3 a	1.6 a	1.6 a

[x]25 μl of spore suspension (10^3–10^4 spores/μl) applied to each root plate.
[y]Infection rated as: 1, symptomless; 2, brown discoloration on area of application; 3, hyphal growth and tissue decay of less than one-fourth of discolored area; and 4, hyphal growth and tissue decay of greater than one fourth of discolored area. Values are means of 10 replicates. Means separation within column by Duncan's multiple range test, 5% level.
[z]Sterile water only applied.

TABLE 2. Ability of four isolates of *Penicillium* spp. to grow on potato-dextrose agar containing fungicides during 4 days at 23–26 C

Fungicide	Rate (μg a.i./ml agar)	Growth ratings[x] for isolate 1[y]	2	3	4
Benomyl	500	++	0	0	0
	1,000	++	0	0	0
	2,000	++	0	0	0
Captan	600	+	+	0	+
	1,200	+	+	0	+
	2,400	+	+	0	+
Prochloraz	150	0	0	0	0
	300	0	0	0	0
	600	0	0	0	0
Etaconazole	60	0	0	0	0
	120	0	0	0	0
	240	0	0	0	0
Control	···	++	++	++	++

[x]Growth rated as: (++) = uninhibited growth equal to control; (+) growth limited to area of suspension application; 0 = no growth. Both replicates of each treatment yielded the same results.
[y]Isolates 1–3 were *P. corymbiferum*, 4 was *P. rugulosum*.

plates after mixed spore inoculation indicated the resistant isolate to be the dominant isolate (Table 3). The only visible disease symptom on etaconazole pretreated basal plates was an occasional browning of the basal plate.

A few symptoms were observed on water pretreated, water inoculated basal plates. These were likely due to natural inoculum embedded in the basal plates that was not destroyed by the chlorine treatment. However, there was significantly more disease on water pretreated basal plates inoculated with the separate isolates of *Penicillium* than on uninoculated. There was no statistical difference in average infection ratings of the resistant versus the sensitive isolate. However, there was significantly less infection from the mixed suspension than from the sensitive. This same trend was evident when equal percentages of the two isolates were reisolated from the water pretreated basal plates inoculated with the mixed spore suspension.

In vitro isolate ethylene production. The benomyl-resistant isolate 1 produced ethylene when grown on PDA, whereas ethylene levels measured in the jars containing the other tested isolates was not significantly different from the controls through 6 days (Table 4). Average ethylene production of the 7-day-old cultures was 1.5 μl/cm^2 of culture surface area per hour. Levels of 1–6% CO_2 and 14–20% O_2 were measured in the jars at the end of the study.

TABLE 3. Infection and pathogen reisolation from excised tulip bulb basal plates 1 wk after pretreatment with benomyl (2,000 μg a.i./ml), etaconazole (240 μg a.i./ml), or water and subsequent inoculation with 25 μl of a spore suspension of benomyl-resistant (R), benomyl-sensitive (S), or mixed isolates (R + S) of *Penicillium corymbiferum*, or with sterile water (W)

Pretreatment	Inoculum[w]	Infection rating[x]	Reisolated colonies ($\times 10^3$)[y]	Reisolation ratio (%R/%S)[z]
Benomyl	W	1.0 a	0	...
	R	1.6 bc	111 cd	100/0**
	S	1.3 ab	17 a	0/100**
	R+S	1.5 bc	84 bcd	96/4**
Etaconazole	W	1.0 a	0	...
	R	1.0 a	14 a	100/0**
	S	1.1 ab	18 a	0/100**
	R+S	1.1 ab	19 ab	67/33 ns
Water	W	1.2 ab	0	...
	R	1.9 cd	73 cd	100/0**
	S	2.2 d	235 d	0.100**
	R+S	1.4 abc	48 abc	48/52 ns

[w] Spore concentrations were statistically equal with 1.7 ($\pm$0.9) $\times 10^4$ for R and 1.6 ($\pm$0.8) $\times 10^4$ for S. The mixed inoculum (R+S) with total spore concentration equal to the individual isolates was prepared by blending equal volumes of R and S.

[x] Infection rated on 1–4 scale (see Table 1). Values are averages of nine points of inoculation on the basal plates. Mean separation within column by Duncan's multiple range test, 5% level.

[y] Colony count based on a per bulb root plate basis. Data analyzed on $\log_{10}$ transformed scale. Mean separation same as infection rating.

[z] Significant difference between % R and % S indicated by paired *t*-test at 1% level (**) or nonsignificant (ns).

TABLE 4. Ethylene accumulated during 6 days at 20 C in jars containing cultures of *Penicillium* spp.[x]

Isolate	Species	Ethylene (μL/L) Day 1	Day 3	Day 6
1	*P. corymbiferum*[y]	5.19	11.10	15.87
2	*P. corymbiferum*	0.01	0.02	0.05
3	*P. corymbiferum*	0.01	0.02	0.02
4	*P. rugulosum*	0.01	0.01	0.02
Control		0.02	0.03	0.03

[x] Cultures were 7 days old and about 3–5 cm in diameter when placed in 473-ml jars.

[y] Values for isolate 1 significantly different from other isolates and control by Mann-Whitney nonparametric statistic (5% level) on all days.

Ethylene production within packages of precooled bulbs. Application of a spore suspension of the benomyl-resistant isolate 1 (ethylene producing) or the benomyl-sensitive isolate 2 (non-ethylene producing) to the basal plates of precooled bulbs before packaging led to similar package ethylene levels through 2 wk of storage at 20 C (Fig. 1). However, during the third week of storage, ethylene levels continued to increase in packages of bulbs inoculated with isolate 1, while ethylene levels declined in those inoculated with isolate 2. The 21-day ethylene level was 0.58 μl/L in packages containing the non-ethylene producing isolate and 1.42 μl/L in the packages containing the ethylene producing isolate. Control package ethylene levels increased from 0.1 μl/L to only 0.3 μl/L during the 3 wk of storage. The ambient air surrounding the packages contained about 0.09 μl/L of ethylene during the study. The ethylene permeation rate of the film used for the packages had previously been measured at 4.8 L (STP) $\times$ atm^{-1} $\times$ day^{-1} $\times$ m^{-2} at 20 C.

By the end of 3 wk, 38 out of 40 total inoculated bulbs had 100% of the basal plate surface covered with *Penicillium* in the packages. However, a small amount of growth of *Penicillium* was also observed on control bulbs. Eleven out of the 20 total control basal plates were completely free of infection, while nine of them had 10% or less of the basal plate surface covered with *Penicillium*. This infection was likely due to naturally occurring inoculum embedded in the basal plates.

DISCUSSION

Two species of *Penicillium*, *P. corymbiferum* and *P. rugulosum*, were isolated that proved to be pathogenic on tulip bulb root plates. *P. corymbiferum* was isolated more frequently from the bulbs than *P. rugulosum*. The isolates differed in virulence. Of the four isolates of *Penicillium* tested, one of the isolates of *P. corymbiferum* was significantly less virulent than the others. When these same four isolates were tested for sensitivity to benomyl, the less virulent isolate proved to be benomyl resistant. All isolates were sensitive to etaconazole and prochloraz in petri plate assays at rates recommended for fungicide application. In other work, these isolates were successfully controlled by etaconazole and prochloraz in tulip bulb inoculation studies (16).

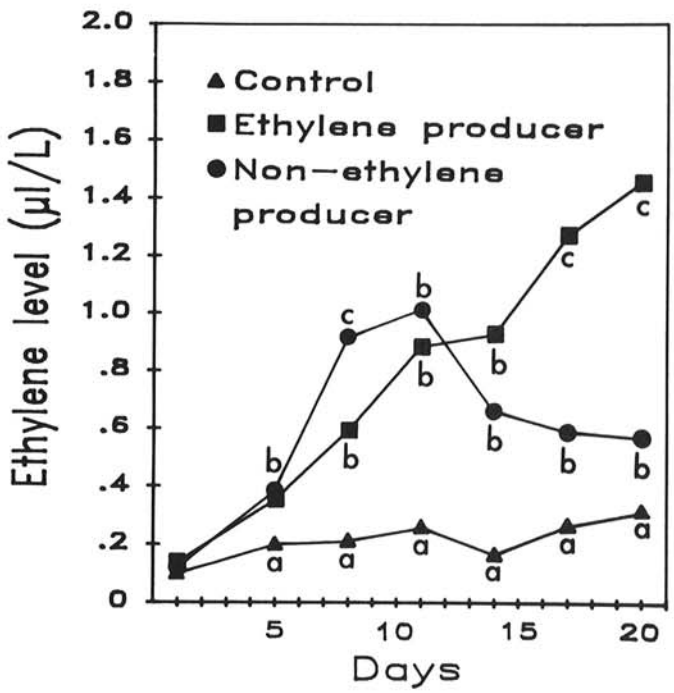

Fig. 1. Ethylene levels in film packages of precooled tulip bulbs through 3 wk at 20 C after basal plate inoculation with an ethylene producing (benomyl-resistant) or a non-ethylene producing (benomyl-sensitive) isolate or with sterile water as a control. Mean separation within sampling date by Duncan's multiple range test at the 5% level.

The isolate competition studies showed that the benomyl-resistant isolate was nearly equally competitive in establishment with the sensitive isolate on water pretreated basal plates (Table 3). This was indicated by the equal percentage of colonies obtained from spores reisolated 1 wk after inoculation with both isolates. In contrast, the pathogenicity study, which rated the ability of the fungus to colonize bulb tissue in a certain time period, seemed to indicate that the resistant isolate was less virulent than the sensitive on nonfungicide pretreated basal plates. This difference suggests that the less virulent isolate may have a slower growth rate. The overall lower infection ratings in the competition study (Table 3) compared with the pathogenicity study (Table 1) were likely due to less protrusion of the root initials from the basal plates caused by the shorter interval between harvest and the beginning of cold storage of bulbs used in the competition study (11).

When both isolates were applied as a mixture to benomyl pretreated root plates, 96% of the spores recovered were of the resistant isolate. Etaconazole pretreatment led to little visible infection and a low number of colonies reisolated, regardless of inoculum applied. The ratio of resistant to sensitive colonies obtained was not statistically different following mixed isolate inoculation. The colonies from all etaconazole pretreatments likely reflected the spores surviving on the surface of the basal plate from the initial inoculation, since no sporulation was visible. These results indicated that etaconazole controlled root plate infection caused by the benomyl resistant isolate of *P. corymbiferum*.

There was significantly less disease and fewer colonies from reisolated spores after mixed isolate compared with sensitive isolate inoculation of water pretreated basal plates (Table 3). This indicates the possibility of inhibition of disease development on the basal plates due to competition between the isolates.

This study suggests that the high levels of infection previously observed on basal plates pretreated with benomyl before packaging (16) may be due to selective growth of a benomyl resistant isolate of *P. corymbiferum*. Other studies have previously reported benomyl resistance in isolates of *P. corymbiferum* and *P. brevicompactum* that are pathogenic to bulbs of the Liliaceae family (3,7,9), so these observations were not unsuspected. The resistant isolate studied here did not grow on PDA containing the experimental fungicides etaconazole or prochloraz. Drenches of these fungicides have also proven effective in controlling the disease on packaged bulbs (16) and in controlling benomyl-resistant isolates of *P. expansum* on stored apples as well (4).

The benomyl-resistant isolate of *P. corymbiferum* was also shown to be an ethylene producer in vitro. Predominance by this isolate could explain the high package ethylene levels observed after benomyl pretreatment of bulbs in previous studies (16). The present study did not eliminate the possibility of ethylene production by the other isolates on different media or during infection of bulb basal plates in the packages (Fig. 1). The sealed film package allowed gas permeation to occur continuously through the film. Therefore, assuming some time for package equilibrium to be obtained, increases in package ethylene level indicated increases in actual ethylene production rate within the package. If ethylene production by both isolates was occurring, then differences in growth rates on the basal plates may have led to the different package ethylene levels during the third week (12). It is also possible that the bulbs themselves produced ethylene in response to infection. The infection of many other plant tissues has been shown to increase host ethylene production (1,20).

Continued use of benomyl fungicide in the normal handling of tulip bulbs may promote the growth of this benomyl-resistant, ethylene-producing isolate. Ethylene exposure of tulip bulbs has been shown to lead to lower levels of tulipalin-A, a naturally occurring fungitoxic substance in bulbs (2). Therefore, production of ethylene by *P. corymbiferum* could ultimately increase basal plate infection by lowering natural bulb resistance. Further studies are needed to determine if other benomyl-resistant isolates are also ethylene producers.

Tulip bulb exposure to ethylene has been linked to many disorders during storage, shipment, and forcing including bulb gummosis, floral shoot rot, poor basal growth, and floral abortion (10). To prevent these problems, the use of benomyl should be discontinued or at least reduced. Other fungicides, possibly etaconazole and prochloraz, should be further tested as possible substitute bulb fungicide treatments.

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