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DESIGN AND FUNCTION OF A MODIFIED ATMOSPHERE PACKAGE

FOR PRECOOLED TULIP BULBS

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DESIGN AND FUNCTION OF A MODIFIED ATMOSPHERE PACKAGE FOR PRECOOLED TULIP BULBS

By

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ABSTRACT

DESIGN AND FUNCTION OF A MODIFIED ATMOSPHERE PACKAGE
FOR PRECOOLED TULIP BULBS

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A prediction method was demonstrated as an aid in selection of a polymeric film for modified atmosphere (MA) packaging of precooled tulip bulbs (Tulipa gesneriana L. 'Kees Nelis'). A small consumer package of 5 precooled (5°C) bulbs sealed in ca. 800 cm^2 of LDF-301 low density polyethylene film obtained equilibrium levels of ca. 5% O_2, 4% CO_2, and 0.1 µl/liter ethylene at 20°. This package maintained excellent bulb flowering ability through 4 wks of storage while non-packaged bulbs flowered poorly. Fusarium oxysporum infection of bulbs of other cultivars yielded package ethylene levels of 2-47 µl/liter, which reduced subsequent flowering. Penicillium was shown to infect the root plate of the packaged 'Kees Nelis' tulip bulbs. Infection was associated with increased package ethylene and CO_2 as well as reduced O_2 levels, and led to reduced rooting and increased floral abortion during subsequent forcing of the bulbs. Prochloraz and vanguard pretreatment of the bulbs prior to packaging controlled Penicillium growth. Benomyl, captan, and chlorine dip pretreatments did not control infection. Three pathogenic isolates

of Penicillium corymbiferum and one P. rugulosum isolate were obtained from the tulip bulbs. One P. corymbiferum isolate displayed benomyl resistance and produced 1.5 $\mu l/cm^2 \cdot hr$ of ethylene when grown on PDA. Vanguard and prochloraz controlled growth of this isolate on PDA. Packages of 'Kees Nelis' bulbs stored at 20° for 1 wk followed by 3 wks of temperature fluctuation between 15 and 25° displayed little change in package CO_2 and O_2 levels. The temperature adaptability appeared due to both changing bulb respiration rates and changing film permeabilities to CO_2 and O_2. Non-packaged 'Kees Nelis' bulbs at 20° lost 45%/35% of scale fresh/dry weight and 38%/20% of floral shoot fresh/dry weight during 4 storage wks. The daughter bulbs within these non-packaged bulbs increased 7-fold in fresh and dry weight. Bulbs in packages yielded little change in fresh or dry weight of any bulb organs.

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Guidance Committee:

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INTRODUCTION

Successful marketing of precooled (5°C) tulip bulbs in consumer packages has not been reported to date. These prepared bulbs could be marketed through mail-order or other retail channels for mid-winter indoor forcing or spring planting in the Northern United States or winter outdoor planting in the South. A marketing period exceeding the 8 days now recommended and maintenance of excellent bulb flowering ability without a refrigeration requirement would be necessary.

Maintenance of flowering ability in these post precooled bulbs has been shown to be enhanced by low O_2 atmospheres. However, normal methods of controlled atmosphere (CA) maintenance would be impractical and expensive during marketing of these bulbs. Since many partially successful modified atmosphere (MA) packages of various commodities have been reported, the objective of this research was to develop and evaluate an MA marketing package for tulip bulbs utilizing a semi-permeable polymeric film for atmosphere maintenance.

A simple prediction method for package parameter optimization was utilized in package development. Disease control, ethylene accumulation, bulb organ fresh and dry matter distribution, and effects of temperature fluctuation, as well as maintenance of bulb flowering ability, were considerations in package evaluation.

LITERATURE REVIEW

This review comprises two sections. The first outlines storage of various commodities in polymeric film packages. Included in this section are prediction methods for package parameter optimization, effects of temperature fluctuation on the package environment, and the development of diseases in packages. The second section outlines tulip bulb morphology and physiology as they relate to bulb storage in various atmospheres.

I. Commodities in Polymeric Films

Polymeric film packaging is used for a wide variety of fruits, vegetables, and ornamentals. Controversy exists as to whether the polymeric films can be used most effectively for perforated packages to aid only in moisture retention of a product, or for sealed packages to create modified atmosphere (MA) conditions.

In early research, Scott and Tewfik (94) found that deleterious high CO_2 and low O_2 levels occurred in sealed cellophane (CP) and pliofilm (PF) packages of tomatoes, snap beans, sweet corn, and apples. All stored for one week or less with poor results. They concluded that different film wraps would be required for each commodity to avoid deleterious effects of gaseous modification unless the consumer packages were perforated. They also reported that existing consumer packages were not airtight, so that deleterious effects were not

occurring in the marketplace. Film perforation was supported by Allen and Allen (4) who found that all films in use in 1950 were not permeable enough to O_2 to be used for sealed market packages. Upon testing 40 films for produce packaging, Schomer (88) found that refrigeration was more important than film type, since all films required perforation to avoid anaerobic effects. One exception noted was cherries, which could be sealed in PF-type FM1-80 without ventilation (32). This film was found to be permeable enough to prevent fogging and deleterious atmosphere modification during a handling and marketing period. Sommer and Luvisi (101) noted the occasional success of a sealed package in delaying fruit ripening, but still recommended perforated packages. In a review of the subject, Hardenburg (37) concluded that, in general, perforation of packages was necessary to eliminate anaerobic respiration, development of off flavors, and swelling due to CO_2 accumulation in the package.

Citrus in particular appears to require ventilation in a polymeric film package. As few as 4 to 8 (107) and as many as 72 ¼" holes (45) per 5 lb polyethylene (PE) bag of oranges have been recommended. Reductions have been observed in weight loss, peel color changes, stem-end rot, and deformation of grapefruit in individual vented PE bags as compared to non-bagged storage (62). Similar wrapping of grapefruit reduced the required relative humidity range during shipment without increasing weight loss (63). This kept fiberboard boxes drier and stronger, which minimized box distortion during handling. In summary, the role of perforated polymeric film packaging in reducing transpirational loss of produce appears well documented.

However, other findings suggest that it is possible to extend applications of polymeric film packaging into the MA realm. These studies involved specific commodities and specific films. One example is the extensive work reported on the development of MA shipping and marketing containers for bananas (65, 89-92, 111, 112).

Scott and Roberts (92) first reported an increase of 6 days in maintenance of the keeping quality of bananas in large PE bags at 20°. Ripening appeared to be significantly delayed by the gaseous atmosphere modification. In further work (91), they found that $KMnO_4$-saturated vermiculite reduced the ethylene levels in the bags and yielded increased firmness of the fruit. The authors suggested that the treated vermiculite absorbed ethylene produced by a few early ripening fruits and thereby inhibited premature ripening. Even though the packaging was successful, large variabilty in CO_2 (1.3 - 20.7%) and O_2 levels (1.2 - 16.3%) occurred, possibly indicating leakage problems. Woodruff (112) demonstrated that 1 ppm ethylene was the threshold level leading to increased banana respiration in PE packages. Accordingly, addition of the ethylene scrubber Purafil to PE bags of bananas was reported to absorb ethylene and extend storage life (65). Oxygen levels in these packages, near 1% by the 8th storage day, were generally lower than measured by Scott and coworkers (91, 92). No anaerobic effects were reported, although the ratio of film surface area to banana weight appeared different from that used by Scott and coworkers, which may have led to lower O_2 levels. However, Scott and Gandanegara (90) reported that PE bagged bananas had increased storage life over a wide range of shipping temperatures, while again reporting a variable gaseous environment. They concluded that a careful

consideration of film surface area to banana weight ratio was not important precisely because bananas respond to a wide range of CO_2 and O_2 levels. This generalization about the commodity/film ratio may not hold for other commodities if a narrower range of MA conditions is necessary for proper storage and/or subsequent ripening.

Packaging of bananas in consumer-size sealed packages also has been investigated (16). Four types of polyvinylchloride (PVC) films were used to overwrap bananas on trays. The most permeable film could extend the shelf-life of bananas to 30 days at 15° by maintaining ca. 3% CO_2 and O_2 in the trays.

Consumer packaging of tomatoes also has been investigated. Tomatoes sealed in many different films showed varied weight losses during storage (7). Cellulose acetate (CLA) was found to yield the best quality tomatoes presumably due to its high permeability to CO_2 and O_2. Off flavors, condensation, and rot occurred in some of the packages, apparently due to anaerobic conditions, although atmospheric levels were not reported. Tomatoes packed in PVC or Haiesu-film (HF) bags kept well for 5 days at 20-24° (78), but subsequently softened, changed color, and produced a fermentation odor. Fruit packaged in PE displayed delayed ripening while the climacteric CO_2 production was reduced by 40% (77). The most successful results with tomatoes were obtained in PVC and PE packages by Saguy and Mannheim (86), who reported satisfactory quality after 21 days at 25° compared to a shelf-life of less than 7 days for controls held in air.

Limited packaging studies with lettuce have been undertaken. Detrimental effects were observed when heads were packaged in PE and

then put under controlled atmosphere (CA) conditions (2.5% CO_2 and O_2) (96). It is possible that anaerobic or high CO_2 effects were present although no internal atmospheric levels were reported. The CA and CA plus packaging conditions did yield higher retention of starch and total sugars than cold storage alone (97). Lettuce in sealed PE bags with and without an initial N_2 flush gave better keeping quality than lettuce stored in air at 1° (1). The N_2 flush gave better results presumably because the O_2 level was reduced faster. Atmospheres reached 2-3% CO_2 and 12-13% O_2 in the bags. The CO_2 appeared to damage crisphead lettuce but not Romaine. Shredded lettuce also has been packaged in sealed film (80). Control PE bags began with air only while others had injections to 30% O_2. The O_2 level fell to 1-2% and CO_2 reached 10% after 1 wk at 2.5°. It appeared that O_2 injection did not greatly affect the time to equilibrium or the ultimate O_2 level obtained.

Other vegetables have been stored in MA packages. Pea pods, kidney beans, lettuce, and bell peppers were found to store better in PE and polystyrene (PS) bags than in other films tested (50). Again, N_2 flushing led to faster atmospheric equilibrium and extended the keeping quality. Carrots stored in sealed PE bags (81) attained levels of ca. 3% CO_2 and 17% O_2 at 10°. Of additional interest was the low phenolic increase in carrots in bags, presumably due to the CO_2 levels and not the relatively high O_2 levels. Similar atmospheric levels were obtained in trays of bell peppers overwrapped with PVC film (11). However, the attributes of this package seemed to be due mainly to reduction of transpiration.

Research in the shipment of pallet loads of strawberries covered with PE film has been reported by Harvey and coworkers (39-41). Additions of CO_2 gas or dry ice in the pallet before shipment were successful with dry ice addition being optimal. The CO_2 suppressed fungus growth and slowed ripening of the berries.

There has been some interest in the use of sealed film packages to reduce chilling injury in citrus. Chilling injury of grapefruit has been reported to be at least partially controllable with high CO_2 levels (106), but less so by low O_2 levels (34). Chilling injury was prevented for 1 month at 4.5° by sealing fruits in containers with PVC and cast vinyl (CV) films (109). It was presumed that the CO_2 levels reduced the symptoms although the increased RH levels could have aided also. No attempt was made in this research to develop a practical package for chilling injury reduction in citrus.

Polymeric film packaging of cut flowers has been investigated as well. Hauge and coworkers (42) used heat sealed CP packages for rose storage at 4-7° for 5 days. Packaged roses had better keeping qualities than non-packaged controls presumably due to CO_2 accumulation (5%) and O_2 depletion (15%). Carnations in the same packages for 8 days lasted 2-3 times longer than controls. Results with pompon chrysanthemums were less successful due to a larger headspace in the package which slowed the CO_2 accumulation.

Other studies utilizing 7 different film types for packaging various cut flowers have been reported (43). Roses stored at 10° and below for 5 days were in good condition in all films except 300 LSAT film (Dupont) which yielded O_2 levels less than 1%. Chrysanthemums

packaged in 300 PMBS film (Sylvania) were stored for periods up to 28 days at 0-10°. The CO_2 levels ranged from 1 to 11% with higher temperatures and longer durations yielding the highest levels. High CO_2 levels were associated with abnormal flowers. Carnations sealed in 300 MSAT film (Dupont) showed little O_2 depletion (17%) or CO_2 accumulation (2%) at 0-10° for 10 days. However, carnation respiration rate was found to increase in the package, most likely due to ethylene accumulation (not reported).

Von Oppenfeld et al. (108) sealed cut tulips in consumer units with PE, saran, CP, and plastic-coated CP. Flower quality was acceptable after storage at 0° for all films except the plastic-coated CP, which yielded CO_2 levels up to 21%. CP film wrapped packages of cut tulips at 5° gave mixed results (76). However, cut tulips kept well for 10 days in laminated PP-CP bags inflated with N_2 and air (5). No package atmospheres were reported, however. PE film packaging of cut roses with initial N_2 flushing kept blooms in the tight bud stage for 40 hrs at 15° (105). The O_2 levels rose from near 0 to 2.5% during the 40 hours.

Attempts at packaging entire plants also have been reported. Foliage, flowering, and bedding plants were packed in 3 mil coextruded PE-PP packs which were sealed and injected with air to form pressurized containers (35). These were held in the dark for 2 days at 16°, then for 30 days under low light. Only purple passion plant (Gynura sarmentosa) stored successfully. The most frequent problem was that plants totally collapsed upon removal from the bags. Since no atmospheric levels were reported, the cause could not be determined. Possibly ethylene, high CO_2, or low O_2 levels were responsible.

Further work with potted foliage plants yielded a good marketable product with a few species after 60 days without a watering requirement (36). Plant collapse was still seen occasionally. Again no package atmospheric data were provided.

Work by French researchers (31, 67) has described the use of silicone rubber (SR) membranes to create controlled atmospheres in storage units of apples and vegetables. Specific PE bags with varied amounts of SR membranes were designed for specific amounts of commodities. Chosen levels of CO_2 and O_2 were obtained within palletized loads. The advantages of this system were that storage rooms were accessible at any time and that possible losses were reduced to individual bags instead of entire storage rooms. Cost was also determined to be 25% cheaper than standard CA storage units. The SR membrane was also used in a circulating system to maintain CA conditions in entire storage rooms by utilizing varied exposures of the membrane.

Recent work has centered upon individual wrapping of fruits and vegetables. Avocados packed individually in PE bags had increased storage life while $KMnO_4$ addition further delayed fruit softening (79). The $KMnO_4$ addition was found to have little effect upon O_2 and ethylene levels, but did appear to decrease CO_2 in the packs (13). Avocados appeared to respond to wide ranges of CO_2 and O_2, a response similar to that noted earlier for bananas.

Citrus fruit individually wrapped in high density polyethylene (HDPE) had twice the storage duration and a 5-fold reduction in weight loss compared to open controls (9). Respiratory activity and ethylene production both were reduced by wrapping, although no differences from controls in internal CO_2 and O_2 levels were noted after 1 month.

Storage of kiwifruit (Actinidia chinensis) in individual PE bags resulted in variable CO_2 levels as well as variable results in flesh softening data (70). Generally, bagged storage yielded less flesh softening than air storage. Kawada (61) reported that individual vacuum film packaging of tomatoes, persimmons, and grapefruit was more effective than non-vacuum packaging in extending storage life. However, the author cautioned that this system was only a supplement to proper refrigeration.

In summary, many perforated polymeric film packages have been shown to function well by reducing transpirational water loss from a commodity. In addition, examples of at least partially successful MA packages have been reported. However, much of this work has been performed with somewhat arbitrarily chosen parameters (i.e. film type, package size, amount of commodity, etc.). It is impossible to estimate how poor choices of packaging parameters have influenced the results of MA packaging efforts. It does appear that definitive and faster results could be obtained if package parameters were optimized before testing. This aspect of packaging deserves futher discussion.

Optimizing Package Parameters. Henig (46) described a produce packaging system as a dynamic one where respiration and permeation are occurring simultaneously. Therefore factors affecting either respiration or permeation rate or both must be considered when designing a package. He identified commodity weight, stage of maturity, membrane permeability, temperature, O_2 and CO_2 partial pressures, ethylene level, light, and possibly other factors as affectors of produce respiration rate. In addition, variables affecting gas permeation

through a film were identified as structure of the film, thickness, area, temperature, and O_2 and CO_2 concentrations. Henig recommended that the package design be directed towards achieving the optimal gaseous composition in a package. He also noted that perforation destroys the semipermeable nature of a film, making gas exchange independent of the chemical nature of the gas or its interaction with a polymer. This results in less control over the package gaseous environment by the package designer.

Tomkins (104) studied the dynamics of a polymeric film package and found that after a period of adjustment, equilibrium levels of CO_2 and O_2 were obtained. At this point, the respiration rate (CO_2 production or O_2 consumption) was assumed to be equal to the permeation rate. If the package was poorly designed, anaerobic conditions were established before equilibrium was obtained. It was concluded that to produce specific package conditions, the correct package size and permeability had to be chosen, and the temperature must be held within fairly narrow limits.

Jurin and Karel (55) studied the package permeation/respiration rate interaction in apple and banana packages. They devised a graphcial solution to predict equilibrium package conditions. The method involved plotting the rate of respiration and permeation at different O_2 concentrations on the same set of coordinates. The equilibrium O_2 and CO_2 concentrations were indicated by the intersection of the curves. These calculations assumed that CO_2 accumulation did not affect the respiration rate and that the RQ was equal to 1. The experimental equilibrium values were in good agreement with the predicted values (ca. 9% O_2 and 3% CO_2).

A detailed computer prediction method for CO_2 and O_2 levels in produce packages has been published (44, 47). To demonstrate this method, tomatoes were enclosed in chambers with known headspaces and film areas. Samples were withdrawn and analyzed periodically for O_2 and CO_2 levels. These values were used to develop regression equations for prediction of O_2 consumption and CO_2 production rates under different O_2 and CO_2 atmospheric concentrations. Two first-order differential equations were developed and solved. This computer-aided iteration technique provided the O_2 and CO_2 concentrations in a package at 1 hour intervals until equilibrium conditions were obtained. Good agreement was found between the experimental and computer calculated results. They also reported that an increase in package headspace only lengthened the time to obtain equilibrium, but did not affect the levels obtained.

From these examples it is apparent that much research time can be saved if some method of predicting the permeability requirements of a film is utilized before any extensive trials are begun. These methods should indicate a range of acceptable film permeabilties for a specific commodity that will yield an acceptable MA package. They may preclude further tests by indicating that with existing films no reasonable film surface area/commodity ratio exists. In this case, prediction methods would at least quantitate the required permeability, with the hope of future film development.

Even with the use of a prediction method, the problems of disease control and temperature fluctuation during marketing and handling remain to be solved for a successful MA packaging system.

Temperature Fluctuation. Tomkins (104) reported that increases in temperature of storage increased the equilibrium CO_2 concentrations within sealed packages. He also noted that temperature decreases could lead to condensation inside the package, which could leach solutes from the produce and support the growth of bacteria and molds. Fisher (29) reported that CP packaging of carnations and pompon chrysanthemums gave good results after 1 month at 0°, but that 7° storage resulted in worthless carnations due to mold development. No package atmospheres were reported in this study, however.

In his review of packaging, Hardenburg (37) displayed data from packages of green beans and the effects of temperature upon the internal package atmosphere. He concluded that temperature had profound effects on package CO_2 and O_2 levels, yielding little hope for practical MA packages. However, it appears that his conclusions were faulty since the packages were not optimized and eventually all would have become anaerobic. Apparent was the effect of temperature upon the rate of obtaining atmospheric equilibrium and not upon the ultimate equilibrium levels.

Other studies have shown varied effects of temperature fluctuation upon MA packages. Roses packaged in various film kept well for 5 days at 0-10° but not at higher temperatures (43). No great atmosperic differences were found between individually sealed PE bags of avocados at 20 or 30° (13). Consumer PVC packages extended the shelf-life of bananas at 15° while odor and taste development was abnormal at 22° (16). The abnormal ripening process appeared to be due to increased CO_2 levels, since no significant O_2 level differences were found. PE bags were found to increase the shipping life of bananas

at 13-37°, although wide ranges of CO_2 and O_2 were reported (90). Henig and Gilbert (47) found that final O_2 and CO_2 levels were nearly the same at 15° and 23° for PVC type RMF-61 and VF-71 film packages of tomatoes. They suggested that temperature changes affected both respiratory activity and film permeability to the same degree with their packages. However, other work with sealed PE bags of head lettuce demonstrated atmospheric changes from 2% CO_2 and 12% O_2 at 1° to 4% CO_2 and 7% O_2 at 20° after only 2 days (1).

The varied effects of temperature upon the success of MA packaging can be explained by the dynamics of a package system itself (46). Temperature change affects both commodity respiration and film permeability. The effect on film permeability is entirely a physical one. Karel (58) has shown by Arrhenius plots that film permeability varies log-linearly with the reciprocal of the absolute temperature and that breaks, or changes of the slope of the line, are rare for films. The effects of temperature on respiration, however, can vary between commodities (30), and depends upon the range of temperatures considered. I have already noted differences in the commodity quality response to various CO_2 and O_2 regimes. It seems apparent, then, that the parameters of film permeability, commodity respiration, and commodity quality response to various atmospheres interact to determine the effect of temperature change upon the success of an MA package. Thus, the effects of temperature change must be evaluated individually for each package commodity system.

Diseases in MA Packages. Various packaging techniques have been shown to decrease/increase disease growth on stored commodities.

Decreases of disease have been mainly due to prevention of cross inoculation by individual wrapping of commodities, while increased disease has generally resulted from the high relative humidity in the package.

Grapefruit that were individually wrapped in non-sealed PE had less stem-end rot than those stored in film-lined boxes (62). It was noted that more calyxes remained green in PE and were thus less likely to become infected. Individual vacuum packaging of other fruits was found to prevent decay organisms from cross-infecting other fruit (61). When adequate fungicides were used, decay also was believed to be low because there was no condensation between the fruit and the tightly wrapped film. Karel (58) has outlined the barrier properties of polymeric films to passage of spores from disease organisms that appear to have functioned in the above studies.

Overwrapping pallet loads of strawberries with PE films and purging with CO_2 gas (10-13%) has led to less Botrytis incidence during transport (40). Transport temperature was ca. 5° and the difference in disease incidence was evident after a subsequent 2 days at 15.5°. Sommer et al. (100) had previously demonstrated the effectiveness of high CO_2 (5-15%) in suppressing strawberry gray mold at 5° or above. However, the common CA conditions of 2-3% O_2 and 5% CO_2 have shown only moderate suppression of most commodity disease organisms (28). Therefore, it is not surprising that in many MA packages, increases of disease incidence have been shown to occur.

Spore germination and invasion of a commodity occurs easily in the near 100% RH of a film package. Therefore, the use of low storage temperatures and wrapping films of high water vapor permeability have been recommended (4). Wounds have been suggested as the main avenue

of disease entrance within packaged commodities (101). Humid atmospheres, while reducing commodity shrinkage, also make an excellent environment for colonization of wounds by pathogens.

Fungicide treatment has controlled disease infection in certain instances. Okubo and Maezawa (78) reported that mold development was sometimes a problem with tomatoes stored for 5 days in PVC and HF bags. Similar mold growth on tomatoes packaged in several PVC and PE packages at 25° was reduced by the use of the most permeable films and by pretreatment of the fruit with 25 ppm chlorine, 1000 ppm Nipagin-M, or 1000 ppm Nipacide (86). Best results were noted with fruit treated with 25 ppm chlorine and wrapped in VF-71 and TPM-87 PVC films. Decay was 0-5% after 21 days at 25°, while control deterioration was 40%.

Disease growth has been a problem in perforated as well as sealed packages. Grierson (33) compared disease growth in mesh vs. perforated PE bags of tangerines and oranges. Results of 35% vs. 55% spoiled packages for tangerines and 3% vs. 10% for oranges were obtained. The use of diphenyl or 2-aminobutane reduced decay in the packages.

Scott and Roberts (93) reported that thiabendazole could control the black-end storage rot of bananas during shipment. However, it was found that the handling method determined the severity of the disease in PE packaged bananas (89). Benomyl or thiabendazole was recommended if the fruits were packaged in PE bags as hands or as single fruits. If they were shipped as a bunch, fungicide treatment was not recommended. Disease entrance through cut tissue of hands or single fruits was involved in the disease severity difference.

Storage rots were also found to be a problem with a small percentage of avocados held in individual PE bags at ambient temperatures

(20-30°) for ca. 10 days. Anthracnose rot occurred in benlate dipped avocados after 40-50 days at 10° in individual PE bags (79). Storage was terminated even though fruits stored with $KMnO_4$ were still firm. Infection was observed mostly at the stem end where the pedicel dried during storage.

Erwinia carotovora was the predominant cause of decay in packaged bell peppers (11). The bacterial soft rot was more severe in PE film packs than in PVC. Overall, however, very little decay occurred at 7, 12, and 25° in the packs.

Mold growth has been noted as a problem in packages of tulips (76, 108), carnations (29), and chrysanthemums (29) in various films. Species of bacteria, as well as Botrytis, Fusarium, and Pythium species, were isolated from flowering pot plants and bedding plants stored in coextruded PE-PP bags (35). It was not determined whether they were pathogenic or secondary invaders, but packaging led to decayed flowers and foliage, making the product unmarketable.

In addition to the commodity destruction and cosmetic damage caused by disease growth in a package, ethylene production by the host and/or the pathogen during disease development can be detrimental. In a study of 228 species of fungi examined in pure culture, about 26% produced ethylene as a metabolic product (52). The host tissue itself has also been shown to produce ethylene upon infection. Williamson (110) was the first to clearly recognize that typical disease symptoms were due to an increase in ethylene production by infected tissues. He reported that high ethylene levels were associated with blackspot of roses (Diplocarpon rosae), chrysanthemum flowers infected with Ascochyta chrysanthemi, Septoria leaf spot of chrysanthemums, and Alternaria leaf

spot of carnations. Cut carnations infected with Botrytis also displayed a marked surge of ethylene production at the onset of fungal attack (98).

Detailed studies demonstrating ethylene production by potato tubers infected with the black rot fungus Ceratocystis fimbriata (12, 53, 102) and tomatoes infected with Fusarium (27) have been published. The soft rot bacterium, Erwinia carotovora also has been shown to induce host ethylene production (66).

All of these studies indicate that commodity infection by disease organisms could lead to ethylene accumulation in an MA package. The level obtained would ultimately depend upon the production rate and the permeation rate through the specific polymeric film being utilized.

II. Tulip Bulb Storage Studies

This section discusses the limited studies of storage of tulip bulbs under various atmospheres. To provide a framework for this discussion, an outline of the tulip bulb life cycle and factors affecting bulb respiration rate and ethylene production rate is first presented.

Tulip Bulb Life Cycle. The tulip bulb life cycle has been outlined by Rees (84). The tulip bulb is composed of concentric scales separated by short internodes. The outer scale, or tunic, is brown and papery. The scales are joined at the basal plate from which emerge the flowering shoot and the roots. Daughter bulbs are initiated in the axils of the scales. Each mother bulb planted in the autumn dies upon flowering and is replaced by daughter bulbs. The initial starch content of the mother bulb scales has been shown to account for only

17% of the starch found in matured daughter bulbs. Thus about 83% of the starch in matured daughter bulbs is derived from photosynthesis before leaf senescence (6). Accordingly, the total dry weight of the daughter bulbs increases rapidly during the spring, with the increase peaking at the time of leaf senescence.

The apices within the daughter bulbs are still in the vegetative state at lifting time in July in the Netherlands. Flower initiation occurs when the bulbs are in storage or during shipment. Differentiation progresses from the tepals to the anthers, being completed with formation of the tri-lobed gynoecium. With the completion of the differentiation process, the bulbs are said to have reached stage G (8).

Low Temperature Requirement. After stage G is attained, a period of low temperature is necessary for normal flower stalk elongation during the spring under natural conditions or during forcing (38, 84). Special precooling (or 5° storage) has been described as storage of tulip bulbs at 5° in a dry, unplanted state to satisfy completely the low temperature requirement before planting (17). This method is in contrast to more standard methods whereby all or part of the cold treatment (generally 9°) is applied to planted bulbs. The rate of shoot growth and number of bulbs flowering has been shown to increase with duration of 5° storage, with 12 to 14 wks duration appearing optimal (74). DeHertogh (17) recommends this duration to forcers in the United States. However, Kawata (60) found that a treatment of 17° for 2 wks plus 5° for 9 wks, or the standard Japanses treatment of 15° for 2 wks plus 2° for 7-8 wks, gave early and high quality

flowers. More recently it has been shown that some -1° treatment may be advantageous (75), although treatment below 5° has sometimes resulted in more blasted flowers upon forcing (60). Additionally, Hoogeterp (51) has suggested a 2° treatment for late forcing only. Thus it appears that the optimal combination of temperature and duration has yet to be established.

Interruption of the precooling after 6 wks by a period of warm storage did not nullify the cold treatment effects for tulip bulbs (85) as has been reported for lily bulbs (69). Such an interruption hastened rather than delayed flowering of tulip bulbs except at 30°, where all of the flowers aborted. This finding indicates that warm temperatures during a post-precooling marketing period before planting may not erase the effects of a previous precooling period.

Bulb Respiration Rate. Studies of bulb respiration rate before, during, and following the cold treatment have been reported. Algera (2, 3) found that after a minimum was reached in mid-August, CO_2 production by tulip bulbs slowly increased. Low temperatures were found to reduce respiration while a return to 20° increased it. Removal of the tunic increased respiration two- to three-fold.

Rees (84) found that after lifting, O_2 consumption was maximal and then decreased to a steady state through October. Bulbs held at 17° through the winter months had low CO_2 production rates until January when they increased (83). This same study showed that bulbs precooled at 5° for 12 wks or longer had higher respiration rates than bulbs precooled less than 12 wks. Since some have considered

this duration optimum, the post-precooling respiratory increase may be associated with completion of the cooling requirement.

Studies of mitochondria isolated from tulip bulb scales subjected to different cold treatments also have been reported. Cooled bulbs showed more active mitochondria than uncooled bulbs when measured as ability to oxidize succinate, malate, or 2-oxoglutarate (49). Arrhenius plots of mitochondrial oxidation in uncooled bulbs showed a single transition point, but bulbs cooled for 8 wks or longer at 2° showed indications of two discontinuities in the diagrams (48). The author suggested that tulip bulbs are chilling sensitive with a phase change occurring in the mitochondrial lipid bilayer during cooling.

In summary, bulb cooling has been shown to increase subsequent isolated mitochondrial as well as whole bulb respiratory activity. This increased activity may be involved in the subsequent shoot elongation and flowering in the tulip.

Ethylene Effects. A review by Kamerbeek and DeMunk (57) lists the major effects of ethylene on the tulip as gummosis of the bulb scales, bud necrosis, flower bud blasting, and morphological changes resembling those caused by unfavorable temperatures.

Fusarium oxysporum tulipae, which may infect young growing bulbs just prior to harvest, has been found to produce ethylene abundantly in vitro (103). This ethylene in turn is involved in bud necrosis, a storage disorder of tulip bulbs, related to bulb mite infestation (20-23, 25). DeMunk (24) also has shown that ethylene exposure during storage before planting resulted directly in flower

bud blasting. The blasting increased with the period before exposure, the storage temperature, the concentration of ethylene, and the exposure period. However, DeHertogh et al. (19) found that 10 of 27 cultivars tested were resistant to the blasting effect of ethylene. Bulbs infected with Fusarium also can cause problems in the greenhouse after precooling and planting. Symptoms include growth retardation and yellowing of the leaves, and death of the plant before flowering (87). Ethylene up to 10 ppm has been measured in the soil atmosphere surrounding bulbs infected with Fusarium (87).

The production of ethylene by healthy, uncooled tulip bulbs was reported as too low for detection in samples of surrounding air by DeMunk (22). However, Prince et al. (83) reported that ethylene production of bulbs kept at 17° fluctuated between 0 and 1 $ml \cdot kg^{-1} \cdot day^{-1}$ until January, after which it increased and became very variable. Moe et al. (71) were the first to report on the ethylene production of precooled bulbs. Production reached a peak 3-4 days after removal of the bulbs from 5° to 21°. One wk later, a second peak related to flower blasting was observed. Increasing the duration of 5° storage increased the ethylene production. Ethylene emanation during 5° precooling also has been reported (83). An initial peak of ethylene production occurred during the second wk of cooling, followed by a major increase after 12 wks. This latter increase may have been related to completion of the cooling requirement.

Storage Studies. The use of low pressure storage of non-cooled tulip bulbs, and the storage of precooled tulip bulbs in various packages, closed systems, and low O_2 ventilated systems has been noted.

Storage of non-cooled tulip bulbs for 14 days in August at pressures of 76 or 150 mm Hg suppressed leaf growth and floral development (18). The treatments delayed flowering after subsequent forcing of most cultivars. The authors concluded that low pressure storage offered no advantage over ventilated temperature-controlled units now being used for shipping. Application of low pressure during other phases of the tulip bulb forcing season has not been investigated.

Various storage methods for bulbs removed from 5° treatment have been studied. Precooled bulbs held in poorly ventilated cardboard boxes for more than 4-8 days at warm temperatures formed many deformed, blasted, or poor quality flowers upon forcing (73). These floral disorders differ from blindness, which results when no floral organs form within the bulb before precooling. Symptoms of floral bud injury were found within bulbs 8 days after removal from 5° to 21°, and increased with temperature, ethylene level, and duration of storage (71, 72). However, bulbs kept in static chambers where atmospheres approached 1% O_2 and 16% CO_2 at 15 or 21° did not show injury. Since a ventilated system with 4.5% CO_2 did not prevent the injury, it appeared that the effect was due to low O_2. From these studies, practical recommendations were made that special precooled bulbs be held at 15° or lower; that maximum ventilation be provided to avoid ethylene damage; and that the shipping period not exceed 8 days.

Prince et al. (82) studied further the effects of low O_2 levels on precooled bulbs. Flowering of 'Kees Nelis' bulbs was not impaired after 4 wks of storage at 17° in either 3 or 5% O_2 in a ventilated system. Air storage in a ventilated system yielded poor flowering. Cultivar and forcing period were limiting factors. Low O_2 storage

was shown to reduce ethylene-induced floral abortion. The primary benefit appeared to be reduction of the post-cooling respiratory rise compared to that of bulbs stored in air. This reduced respiration effect has been reported for numerous other commodities (10, 14, 15, 54, 56, 59, 64, 68, 95, 99). The favorable results of these experiments led to the studies reported in this dissertation.

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SECTION I

DESIGN OF A MODIFIED ATMOSPHERE PACKAGE FOR MARKETING OF PRECOOLED TULIP BULBS

Abstract. A simple prediction method utilizing mathematical descriptions of bulb respiration and film permeation was demonstrated as an aid in selection of a polymeric film for modified atmosphere (MA) packaging of precooled (5°C) tulip bulbs (Tulipa gesneriana L. 'Kees Nelis'). The method predicted that a small consumer package of 5 precooled bulbs sealed in ca. 800 cm^2 of LDF-301 low density polyethylene film (Dow Chemical) would obtain atmospheric equilibrium at 3-5% CO_2 and O_2 while maintaining normal bulb flowering ability. Two other films, LDF-550 and PSD-599, were predicted as unsuccessful for a package. Packaging trials verified the predictions. Bulbs packaged in LDF-301 film for 3 wks at 20° yielded 70% normal flowers upon forcing. Bulbs in the other films and non-packaged bulbs flowered poorly. Penicillium infection of the bulbs increased CO_2 and decreased O_2 levels in the package, and also decreased the flowering percentage. The package atmosphere appeared to protect the bulbs from package ethylene accumulations to 0.5 µl/liter. Tepal length, plant height, and bottom and top internode lengths upon flowering of bulbs held in the LDF-301 film packages for up to 3 wks at 20° were similar to those from bulbs planted immediately after precooling.

The marketing of precooled bulbs through mail-order or other retail channels for mid-winter indoor forcing in the northern United States or outdoor planting in the South has not been investigated. DeHertogh (5) describes 5° precooling as storage of tulip bulbs in open tray cases at 5° to satisfy all of the bulb cold requirement (8, 24) before planting. These bulbs could offer consumer convenience since no cold treatment would be required for flowering.

Current recommendations suggest that these bulbs be held at 15° or lower, that maximum ventilation be provided, and that a shipping period not exceed 8 days (16). Some storage at warmer temperatures has been attempted. Moe and Hagness (18) showed that precooled bulbs held in poorly ventilated cardboard boxes for more than 4 to 8 days formed many deformed, blasted, or poor quality flowers upon forcing. Moe et al. (16, 17) found symptoms of floral bud injury within tulip bulbs 8 days after removal from 5° to 15° or 21° environments. The symptoms increased with temperature, ethylene concentration, and duration of storage. Addition of 4.5% CO_2 in a ventilated system did not prevent injury, but storage in static chambers where the O_2 content reached 1% did prevent injury. Subsequently, Prince and coworkers (22) found flowering of 'Kees Nelis' bulbs to be unimpaired through 4 wks of storage at 17° in either 3 or 5% O_2 in ventilated systems, while air storage yielded poor flowering. Low O_2 storage also was found to reduce ethylene-induced floral abortion and to reduce the post-cooling respiratory rise compared to that of bulbs stored in air.

Since low O_2 appeared to offer advantages, a practical method for obtaining this condition during shipping and marketing needed to

be investigated. One such method is the use of sealed polymeric film packages. Beneficial effects from this method have been reported for many commodities (4, 7, 9, 15, 20, 27).

Henig (11) has described a produce packaging system as a dynamic one where respiration and permeation are occurring simultaneously. Various methods of predicting the optimal film parameters have been published. Jurin and Karel (13) devised a graphical solution to predict equilibrium package conditions. A more complex computer aided method (10, 12) has utilized differential equations that were developed and solved to predict package atmospheric levels.

The objective of this research was to develop and evaluate a consumer size polymeric film package for marketing precooled tulip bulbs under low oxygen regimes without a refrigeration requirement. The application of a simple prediction method for package parameter optimization is demonstrated. Subsequently, the effects of specific polymeric films upon package gaseous atmospheres and the resultant maintenance of bulb flowering ability are outlined.

Materials and Methods

This research was performed during the 1979-80 and 1980-81 forcing seasons. Tulip bulbs (12-14 cm in circum.) were shipped to East Lansing, Michigan, from the Netherlands in open tray cases in temperature controlled containers at 17-20°. The shipping/arrival dates were Aug. 17/Sept. 7, 1979, and Sept. 10/26, 1980. All bulbs had reached stage G upon arrival and were stored at 13° until the 5° precooling period began.

Package Parameter Optimization. A simple method of package parameter optimization (S. Gyesly, personal communication) was used during the first season. 'Kees Nelis' bulbs were precooled in open tray cases at 5° (± 0.5°) from Nov. 11 to Feb. 7 (13 wks). At the end of the precooling period, one or two bulbs were placed in 473 ml canning jars and sealed with ca. 40 cm^2 of one of each of four selected polymeric films. The films were sealed on the jars with stopcock grease, rubber O-rings, and the jar bands. Three jars (reps) were used for each film/bulb number treatment combination. Internal headspaces were ca. 400 ml (1 bulb) or ca. 320 ml (2 bulbs). The jars were placed randomly in a 20° room. The CO_2 and O_2 levels were monitored over a 15 day period by withdrawing a 2 ml sample through ports of silicone rubber caulking (Dow Chemical) applied to the film surface. Gas chromatography analysis was on a Carle GC-8700 equipped with a thermal conductivity detector.

Four types of polymeric films with widely varied permeabilities were obtained from the School of Packaging at Michigan State University for sealing the jars. The films' permeabilities were measured with the Oxtran 100 (O_2) and Permatran (CO_2) devices (Modern Controls, U.S.A.) in liter$\cdot m^{-2} \cdot atm^{-1} \cdot day^{-1}$ at 20-23°. The films and their O_2/CO_2 permeabilities (± 10%) respectively were: Mylar (0.08/0.20); polypropylene (2.8/3.3); polyethylene (6.5/31.0); and pliofilm (28.0/102.0). All were 0.025 mm thick except the Mylar, which was 0.013 mm thick.

The changes in jar headspace CO_2 and O_2 quantities between each sampling were calculated. These changes were assumed to be simultaneously a function of bulb respiratory activity and CO_2 and O_2

permeation through the films. Therefore, the following steady-state equations were used to estimate the CO_2 emanation and O_2 consumption of the bulbs between each sampling:

$$\frac{\Delta CO_2^H}{\Delta t} = [E_{(CO_2)} - P_{(CO_2)}[pCO_2]], \quad (1)$$

where ΔCO_2^H = change in headspace CO_2 between samplings in $ml \cdot bulb^{-1}$,

$E_{(CO_2)}$ = bulb CO_2 emanation in $ml \cdot bulb^{-1} \cdot day^{-1}$,

$P_{(CO_2)}$ = CO_2 permeation through each selected film in $ml \cdot atm^{-1} \cdot day^{-1}$,

pCO_2 = average CO_2 partial pressure (atm) in headspace during interval t,

Δt = interval in days, and

$$\frac{\Delta O_2^H}{\Delta t} = [P_{(O_2)}[0.21 - pO_2] - C_{(O_2)}], \quad (2)$$

where ΔO_2^H = change in headspace O_2 between samplings in $ml \cdot bulb^{-1}$,

$C_{(O_2)}$ = bulb O_2 consumption in $ml \cdot bulb^{-1} \cdot day^{-1}$,

$P_{(O_2)}$ = O_2 permeation through each selected film in $ml \cdot atm^{-1} \cdot day^{-1}$,

pO_2 = average O_2 partial pressure (atm) in headspace during interval t,

Δt = interval in days.

$P_{(CO_2)}$ and $P_{(O_2)}$ were calculated from the known permeabilities of the four films and the film surface area available for permeation (40 cm^2). The ambient conditions were measured and found to be ca. 0.21 atm O_2 and 0.0 atm CO_2.

These two equations were solved for $E_{(CO_2)}$ and $C_{(O_2)}$ yielding:

$$E_{(CO_2)} = \frac{\Delta CO_2^H}{\Delta t} + P_{(CO_2)}[pCO_2], \text{ and} \quad (3)$$

$$C_{(O_2)} = P_{(O_2)}[0.21 - pO_2] - \frac{\Delta O_2^H}{\Delta t} . \quad (4)$$

The $E_{(CO_2)}$ and $C_{(O_2)}$ of the bulbs were thus calculated between each sampling period. Since the average CO_2 and O_2 partial pressures external to the bulbs for each sampling interval were also calculated, regression equations relating the $E_{(CO_2)}$ and $C_{(O_2)}$ of the bulbs to levels of CO_2 and O_2 external to the bulbs were developed, such that:

$$E_{(CO_2)} = f[CO_2][O_2], \text{ and} \quad (5)$$

$$C_{(O_2)} = f'[CO_2][O_2], \quad (6)$$

where $[CO_2] = 100(pCO_2)$,

$[O_2] = 100(pO_2)$, and

f and f' yielded units of $ml \cdot bulb^{-1} \cdot day^{-1}$.

The polynomial functions f and f' were fitted using the stepwise multiple regression program of the STAT IV statistical package. Each variable up to the third order was added if it was significant at the 10% level. For simplicity, the independent variables were entered as 100 times the partial pressures (e.g. 0.10 atm was entered as 10).

These equations then allowed the calculation of the desired CO_2 and O_2 permeation for an optimized package. It has been demonstrated that at package equilibrium, the $E_{(CO_2)}$ and $C_{(O_2)}$ of a commodity are equal to their corresponding film permeation rates (26).

Therefore, two equations described the relationship between $E_{(CO_2)}$ or $C_{(O_2)}$ and film permeation at equilibrium of an optimized package:

$$E_{(CO_2)} = P^X_{(CO_2)}[pCO_2], \text{ and} \quad (7)$$

$$C_{(O_2)} = P^X_{(O_2)}[0.21 - pO_2], \quad (8)$$

where $E_{(CO_2)}$ or $C_{(O_2)}$ = bulb CO_2 emanation or O_2 consumption at package equilibrium in $ml \cdot bulb^{-1} \cdot day^{-1}$,

$P^X_{(CO_2)}$ or $P^X_{(O_2)}$ = required CO_2 or O_2 permeation of package at equilibrium in $ml \cdot bulb^{-1} \cdot day^{-1} \cdot atm^{-1}$, and

pCO_2 or pO_2 = desired package CO_2 or O_2 partial pressure (atm) at equilibrium.

Equations 7 and 8 applied only if CO_2 and O_2 partial pressures outside of the package were equal to ca. 0.0 and 0.21 atm respectively. Algebraic substitution then yielded:

$$f[CO_2][O_2] = P^X_{(CO_2)}[pCO_2], \text{ and} \quad (9)$$

$$f'[CO_2][O_2] = P^X_{(O_2)}[0.21 - pO_2]. \quad (10)$$

Desired CO_2 and O_2 levels for an optimized package were then substituted into both sides of equations 9 and 10, which then were solved for $P^X_{(CO_2)}$ and $P^X_{(O_2)}$. The number of bulbs per package, as well as a practical package size (film surface area), were decided upon before film selection. This allowed calculation of the required film permeability for an optimized package as follows:

$$P^Y_{(CO_2)} = \frac{P^X_{(CO_2)}[B]}{SA}, \text{ and} \quad (11)$$

$$P^Y_{(O_2)} = \frac{P^X_{(O_2)}[B]}{SA}, \quad (12)$$

where $P^{Y}_{(CO_2)}$ or $P^{Y}_{(O_2)}$ = required film CO_2 or O_2 permeability in $ml \cdot atm^{-1} \cdot day^{-1} \cdot m^{-2}$,

$P^{X}_{(CO_2)}$ or $P^{X}_{(O_2)}$ = required CO_2 or O_2 permeation of package at equilibrium in $ml \cdot bulb^{-1} \cdot day^{-1} \cdot atm^{-1}$,

B = desired number of bulbs in package, and

SA = package film surface area (m^2).

Films with specific manufacturer's numbers were selected for the packaging trials so as to insure repeatability.

Bulb Packaging. Packaging trials were performed during the second forcing season using three films chosen according to the method outlined above. Films were selected for a package of ca. 0.08 m^2 of film surface area (20 x 20 cm, top and bottom) to contain 5 bulbs. This package size was selected to allow for insertion of the bulbs into a prototype plastic holding device before sealing, for subsequent marketing studies. This device is part of a hydroponic forcing pot for tulip bulbs designed for home forcing of bulbs (Netherlands Patent 14.28.24).

The permeabilities of the films were measured with a custom-made stainles steel permeability cell to yield more precise measurements than those obtained from perviously-noted industrial equipment. This permeability cell was divided into 2 sections by ca. 44 cm^2 of the selected film and sealed with stopcock grease and 2 rubber O-rings which were tightened above and below the plane of the film. This created 2 sections of ca. 60 cm^3, separated by the film. Each section was equipped with inlet, outlet, and sampling ports. Through the top section pure CO_2, pure O_2, or 7000 ppm ethylene in air was passed to

yield at least 1 air exchange/min. Pure N_2 at 8-15 ml/min was passed through the bottom section, which was then sampled for CO_2, O_2, or ethylene analysis until a constant value was obtained. 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 detector respectively. The permeability of the films to each gas then was calculated. Three separate determinations were made for each film at 20°.

'Kees Nelis' bulbs were precooled at 5° and 80-90% RH from Oct. 21, 1980, to Jan. 23, 1981 (13.5 wks) for the packaging trial. At the end of the precooling period, 5 bulbs were heat sealed inside each polymeric film package. The resultant package headspace was determined to be ca. 500 ml. Any bulbs with desiccated root plates, a disorder that occurs with a small number of precooled bulbs, were not used in the packages. Four packages (reps) of each film/duration treatment combination were placed randomly into each of two rooms at 20° (35-55% RH) and 25° (20-35% RH). Non-packaged control bulbs were also stored in the open at each temperature. Four replicates of initial post precooled control bulbs were also planted at the start of the experiment to determine if any disorders existed in the bulbs prior to packaging.

The CO_2, O_2, and ethylene levels in the 4 wk duration packages were monitored during the 24 days by withdrawing a 2 ml (CO_2 and O_2) or 1 ml (ethylene) sample through ports of applied silicone rubber for gas chromatography as previously described. At the end of the 2 and 3 wk storage periods, bulbs were removed from the packages for forcing in the greenhouse.

Upon opening the packages, the tunics were removed from the bulbs to facilitate rooting (5). The bulbs were planted 5/pot in 15 cm pots containing 20% vermiculite, 20% perlite, and 60% peat (VSP-Mix, Michigan Peat Company). After planting, each pot was drenched with 0.2% benomyl and placed randomly on the greenhouse bench with a minimum night temperature of 16-17°. The plants were fertilized once with 20N-8.6P-16.6K at 200 ppm N when the roots first reached the bottom of the pots.

When two-thirds of the flower bud developed color, a plant was considered to have flowered. The days to flower, tepal size, plant height from nose of bulb to top of flower, as well as the bottom and top internode lengths of each normal flower were recorded. The average observation from the normal flowers as well as the percent normal flowers then were calculated for each pot (rep).

Statistical Analysis. Stepwise multiple regression was used in the prediction experiment as previously described. Analysis of variance was performed on the package trial data where possible. No statistical comparison was valid between the two temperatures, nor between the two storage durations, due to possible confounding by changing daytime greenhouse conditions during subsequent forcing of the bulbs. The percent normal flower data were based on a small sample (5 bulbs) and did not fit the assumptions of the analysis of variance. For this reason, the Kruskal-Wallis non-parametric 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 distribution assumptions.

Results and Discussion

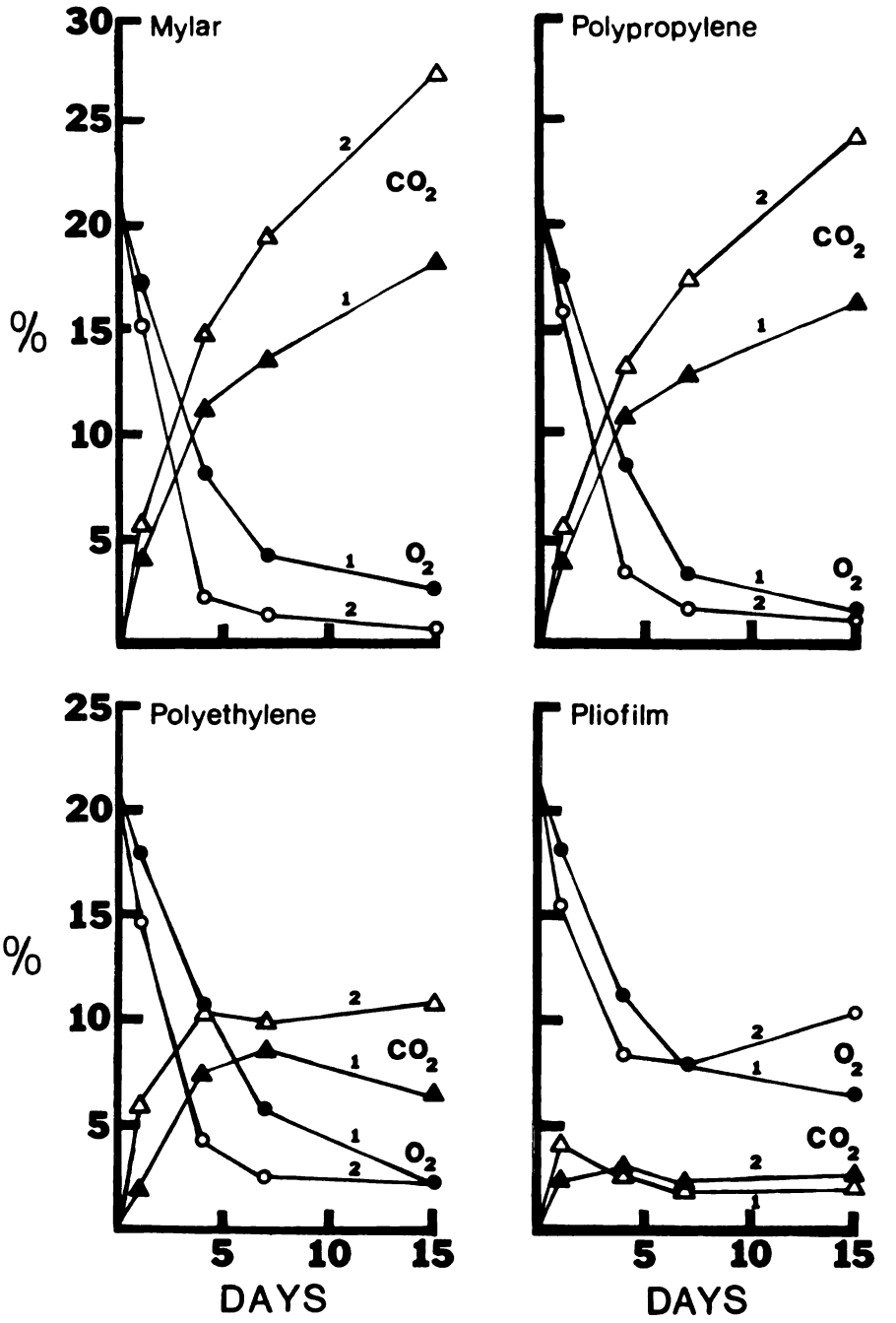
The results of the package optimization study are shown in Figure 1. The trends in the CO_2 and O_2 levels in the jars over the 15 days reflected the relative permeabilities of the 4 films utilized. Jars containing 2 bulbs obtained higher CO_2 and lower O_2 levels than those containing 1 bulb, due to the greater amount of respiring tissue. The various film/bulb number combinations resulted in the bulbs being exposed to varied levels of CO_2 and O_2. This was desirable for developing the prediction equations. The pliofilm sealed over 2 bulbs stretched during the experiment. This altered the film permeation during the study and led to an O_2 increase at day 15. These data were excluded from the regression analysis. The two equations obtained from the multiple stepwise regression were:

$$f[CO_2][O_2] = 9.1 - 0.18[CO_2] + 0.059[CO_2][O_2] - 0.008[CO_2]^2[O_2] \text{ with } R^2 = 0.81, \text{ and} \quad (13)$$

$$f'[CO_2][O_2] = 15.1 - 2.89[CO_2] + 1.01[O_2] + 0.176[CO_2]^2 - 0.003[CO_2]^3 - 0.003[O_2]^3 \text{ with } R^2 = 0.88. \quad (14)$$

These equations did not elucidate any biological relationship between CO_2 or O_2 levels external to the bulbs and bulb respiration, but defined the best fit expressions for prediction purposes only. These 2 functions were substituted into equations 9 and 10 to allow calculation of $P^X_{(CO_2)}$ and $P^X_{(O_2)}$. A range of 3 to 5% O_2 and CO_2 was selected as desirable for the package since earlier research had indicated that these ranges could maintain bulb flowering ability (21, 22). Substitution of this range into equations 9 and 10 yielded $P^X_{(CO_2)}$ and $P^X_{(O_2)}$ ranges of 200-330, and 42-81 $ml \cdot bulb^{-1} \cdot atm^{-1} \cdot day^{-1}$ respectively. These

Figure 1. CO_2 and O_2 levels in jars containing 1 or 2 precooled tulip bulbs and sealed with four different film types. (Values are means of 3 replications.)


Mylar
Polypropylene
Polyethylene
Pliofilm
%
30
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CO_2
O_2
1
2
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15
DAYS

values and the desired package surface area (0.08 m^2) to contain 5 bulbs then were substituted into equations 13 and 14 to yield $P^{Y}_{(CO_2)}$ and $P^{Y}_{(O_2)}$. Package headspace was not considered a critical parameter for the prediction since studies have indicated that headspace affects only the time to obtain equilibrium, but not the ultimate equilibrium levels (12). Substitution yielded final predicted permeability ranges of 12-26 and 2.6-5.1 liters$\cdot m^{-2} \cdot atm^{-1} \cdot day^{-1}$ for CO_2 and O_2 respectively.

Dow Chemical supplied 3 films with permeabilities near these requirements. All were types of low density polyethylene with good heat-sealable properties. The film permeabilities are listed in Table 1. The permeabilities to CO_2 and O_2 of the LDF-301 film appeared to be near the middle of the predicted required ranges. It appeared that the LDF-550 film would be marginally effective due to an O_2 permeability near the acceptible low limit and a CO_2 permeability below the limit. The PSD-599 film had permeabilities too low for both gases. While no prediction for an ethylene permeability range was made, the levels were measured and found to be similar to O_2 permeability for the 3 films.

Packaging Experiment. The CO_2 and O_2 levels through 24 storage days at 20° and 25° are depicted in Figure 2. Ambient conditions in the storage rooms during the experiment were ca. 0.15% CO_2 and 20.5% O_2. Equilibrium O_2 levels in the 2-3% range were obtained in the LDF-550 and PSD-599 packages at both temperatures, with 25° yielding a faster decrease to equilibrium. These levels were lower than the predicted desired range. The LDF-301 packages at 20° appeared to level off at 5% O_2, but subsequently O_2 fell to less than 3% sometime

Table 1. Permeabilities to CO_2, O_2, and ethylene of 3 low density polyethylene films utilized for packaging precooled tulip bulbs.[z]

Film type	Thickness (mm)	Permeability (liter·atm^{-1}·day^{-1}·m^{-2})[y]		
		CO_2	O_2	C_2H_4
LDF-301	0.051	16.43 (0.53)	4.17 (0.12)	5.21 (0.03)
LDF-550	0.076	7.30 (0.12)	2.21 (0.09)	2.55 (0.08)
PSD-599	0.152	4.30 (0.07)	1.43 (0.05)	1.09 (0.06)

[z]Films supplied by Dow Chemical, U.S.A.

[y]Values are means (± 1 SD) of three determinations at 20°C.

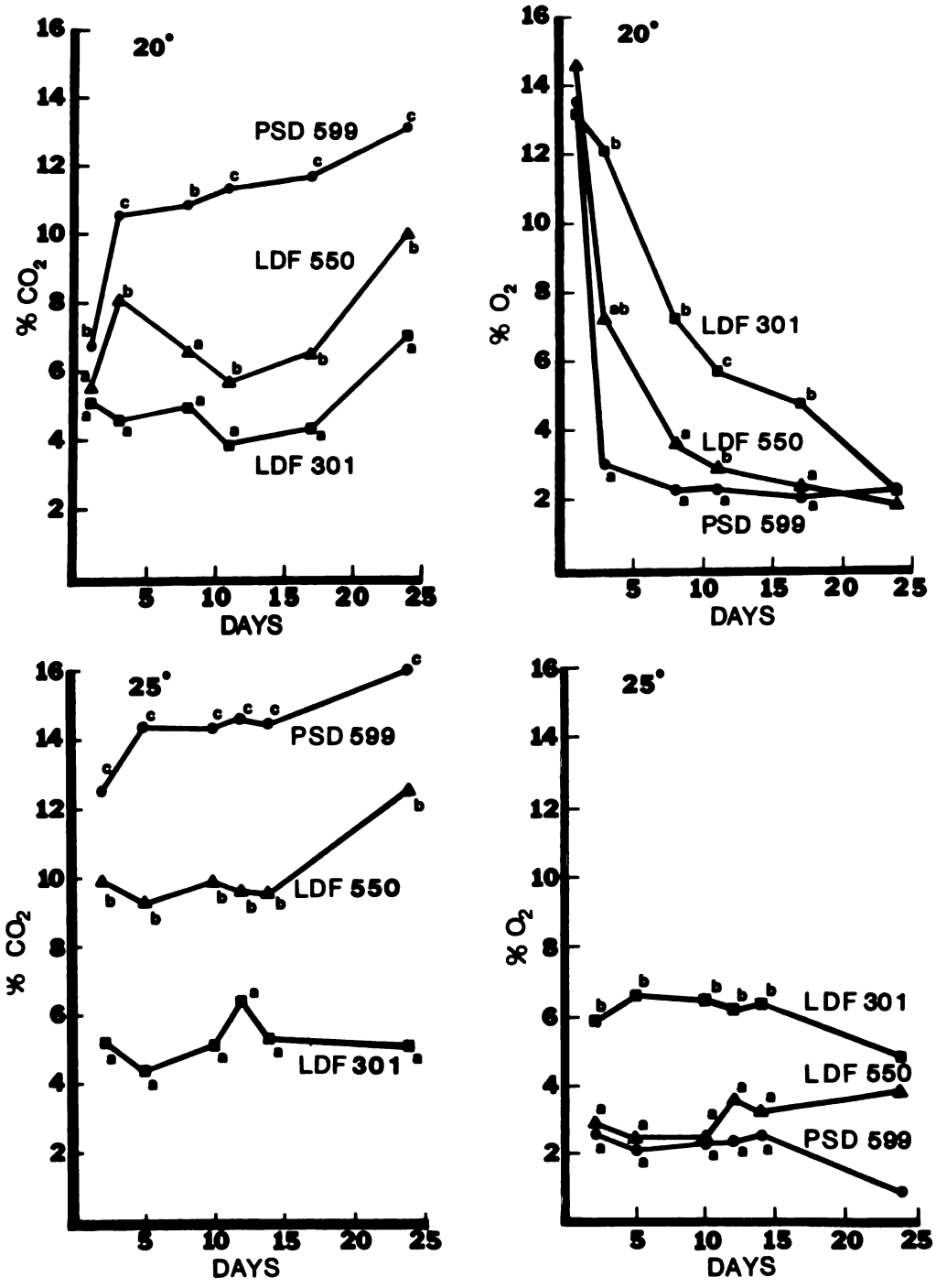

Figure 2. CO_2 and O_2 levels in 3 low density polyethylene packages of precooled 'Kees Nelis' tulip bulbs during 24 days of storage at 20° and 25°C. (Values are means of 4 replications. Mean separation within sampling date by Duncan's multiple range test, 5% level. Absence of letters indicates no significant differences on that sampling date.)

between day 17 and day 24. This decrease, as well as decreased O_2 levels in other packages between day 17 and day 24, was likely due to increased respiratory activity in the packages due to infection of the bulb root plates by Penicillium. This decrease was eliminated when Penicillium was controlled in later packaging studies (Section II). The equilibrium O_2 levels in LDF-301 packages at 25° were near 6%. This was most likely due to increased film permeation at 25°. The effects of temperature change upon the function of the package were subsequently investigated in more detail (Section III).

Levels of CO_2 in the 4-5% range were obtained in the LDF-301 packages at 20° with an increase at day 24, again probably due to Penicillium infection of the bulbs. Both LDF-550 and PSD-599 packages resulted in CO_2 levels above the 5% level. These high levels have previously been associated with "stem topple", a disorder where the tulip stem collapses just at flowering (21). Storage at 25° appeared to result in higher CO_2 levels than 20° storage for LDF-550 and PSD-599 packages, with little difference occurring for the LDF-301 packages.

Ethylene levels in the packages (Figure 3) were variable and were apparently the result of production by the bulbs in response to precooling (15, 23) and as a result of Penicillium infection (Sections II and IV). Ambient ethylene levels were ca. 10 nl/l during the experiment.

The bulb flowering percentages, after storage and forcing, are shown in Table 2. Non-normal flowers displayed varied degrees of floral abortion and shoot growth retardation. These disorders were quantified in later studies (Sections II and III). After 2 wks at 20°,

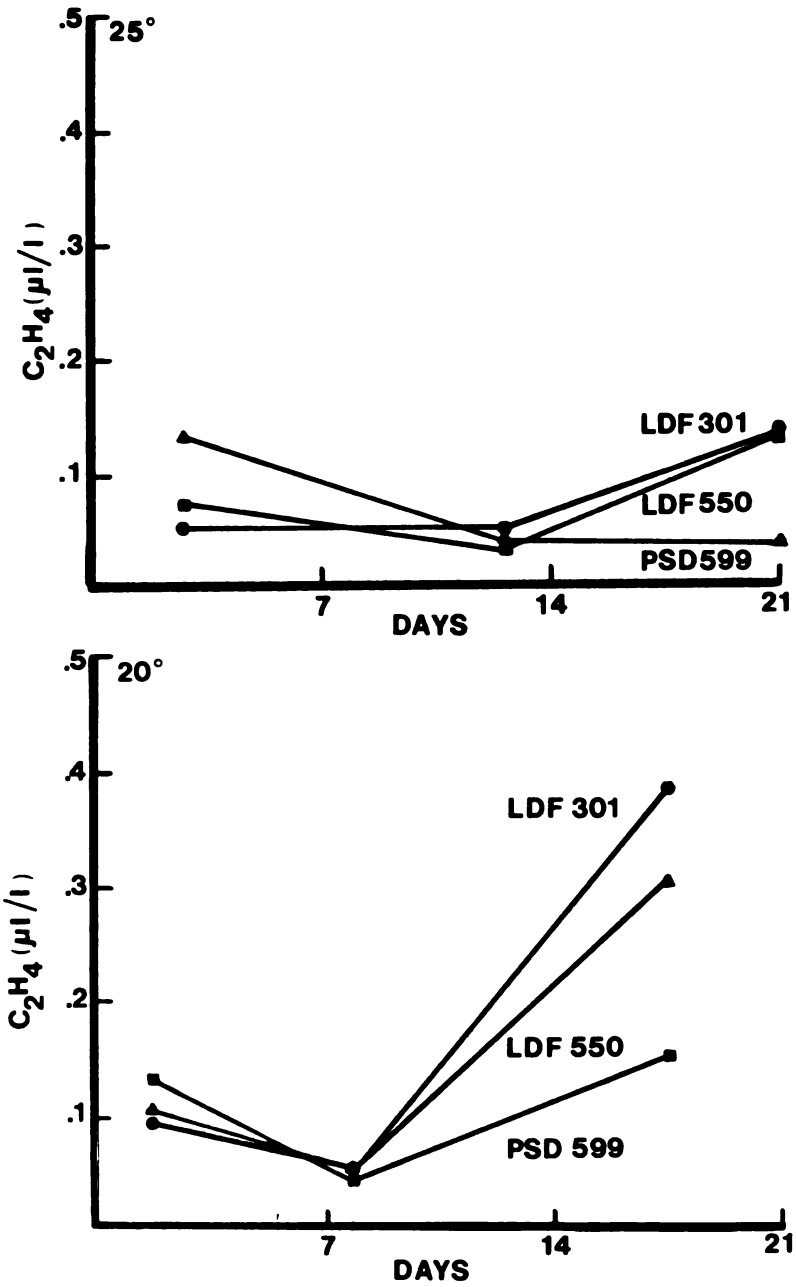

Figure 3. Ethylene levels in 3 low density polyethylene film packages of precooled 'Kees Nelis' tulip bulbs during 24 days of storage at 20° and 25°C. (No significant differences within sampling dates.)

Table 2. Flowering obtained from packaged and non-packaged post precooled 'Kees Nelis' tulip bulbs stored at 20° and 25°C for 2 and 3 wks.[z]

Film type	% normal flowers[y,x]			
	20°		25°	
	2 wks	3 wks	2 wks	3 wks
LDF-301	75a	70b	85b	45b
LDF-550	80a	5a	20ab	0a
PSD-599	55a	0a	0a	0a
Non-packaged	70a	0a	35ab	0a

[z]Initial post precooled bulbs yielded 95% normal flowers.

[y]Means of 4 replications of 5 bulbs

[x]Mean separation within columns by the Mann-Whitney non-parametric test, 5% level.

no significant differences in flowering were visible between treatments. All yielded between 55 and 80% normal flowers, including the open controls. This level of flowering from non-packaged bulbs was better than levels previously reported from bulbs stored in poorly ventilated cardboard boxes (18). The ventilation of endogenous ethylene by open storage alone appeared to lessen floral abortion. Cultivar response differences also could have been involved here (6).

The LDF-301 packages maintained a high level of flowering after 3 wks at 20°, compared to negligible flowering for all other treatments. Penicillium infection of the bulbs was observed in all packages and was believed responsible for reduced flowering from the bulbs in LDF-301 compared to initial post precooled controls. Representative pots of forced bulbs from these treatments are shown in Figure 4. Flowering of bulbs after 2 wks in LDF-301 packages at 25° was 85%, while large variability in the flowering response was observed from LDF-550 packages and non-packaged storage at the same temperature and duration. The LDF-301 packaging for 3 wks at 25° maintained some degree of flowering, but was less successful than at 20°. However, while high temperatures may limit the package success, continuous 25° in a marketing environment is unlikely. The LDF-301 package has subsequently been demonstrated to be adaptable to ambient temperature fluctuation between 15° and 25° with only small changes occurring in the package environment, and with good maintenance of flowering ability (Section III). The poor performance of bulbs from LDF-550 and PSD-599 packages after 2 wks at 20°, and after 2 or 3 wks at 25°, was likely due to low O_2 and/or high CO_2 effects. Because of these poor results, only LDF-301 film was used in further studies.

Figure 4. Representative pots of forced 'Kees Nelis' tulip bulbs after 3 wks of storage at 20°C. (Left to right: non-packaged, in LDF-301, LDF-550, and PSD-599 packages.)

The forcing characteristics of the normal flowers obtained from packaged and non-packaged bulbs are shown in Table 3. The LDF-301 packages maintained most characteristics at similar levels to initial post precooled bulbs through 3 wks. After 2 wks at 20°, non-packaged bulbs yielded lower values than bulbs from LDF-301 packages for total plant height and for bottom and top internode lengths. This indicated some degeneration of the non-packaged bulbs after 2 wks that was not apparent from the percent normal flowering data. Studies have shown that both the leaves and the floral organs provide auxin-like substances which control the elongation of the floral shoot (25), with the gynoecium exerting the greatest control over the top internode (19). Endogenous bulb ethylene levels may have inhibited polar auxin transport (2) in the floral shoot yielding reduced elongation from open stored bulbs. The LDF-301 package may have preserved the normal hormonal status of the shoot by the antagonistic effects on ethylene action of reduced O_2 and elevated CO_2 levels (1, 3), even though some package ethylene accumulation was evident. Previous research has demonstrated that the bulbs could tolerate these ethylene levels if exposed to a 3-5% O_2 atmosphere, without exhibiting a great degree of floral bud blasting upon forcing (22). Bulbs subjected to any post precooling storage flowered in less days than initial post precooled control bulbs, with open stored bulbs flowering in less days than any of the packaged bulbs after 2 wks at 20°. This indicated some shoot development in open stored bulbs that was slowed by the package atmosphere.

Table 3. Forcing characteristics of packaged and non-packaged post precooled 'Kees Nelis' tulip bulbs stored at 20° and 25° for 2 and 3 wks.[z]

Film type	Tepal size (cm)	Plant size (cm)	Bottom internode (cm)	Top internode (cm)	Days to flower
		2 wks/20°[y]			
LDF-301	5.0b	36.5b	9.0b	11.8b	18.6b
LDF-550	4.8ab	33.4a	8.5b	10.2b	19.2bc
PSD-599	4.4a	34.8ab	9.6b	11.0b	20.6c
non-packaged	4.8ab	27.8a	5.7a	8.2a	16.6a
		3 wks/20°[x,w]			
LDF-301	4.7 (0.2)	35.0 (1.7)	8.8 (0.4)	11.5 (0.3)	17.8 (1.0)
		2 wks/25°[x,w]			
LDF-301	4.7 (0.1)	36.0 (1.3)	8.5 (0.6)	12.6 (0.9)	19.5 (0.6)
		3 wks/25°[x,w]			
LDF-301	4.6 (0.2)	30.4 (2.2)	7.3 (0.3)	10.4 (1.6)	19.3 (1.3)

Table 3 (cont'd.).

Film type	Tepal size (cm)	Plant size (cm)	Bottom internode (cm)	Top internode (cm)	Days to flower
		Initial post precooled[W]			
	5.0 (0.1)	33.9 (2.3)	9.0 (0.7)	10.3 (3.2)	23.9 (0.6)

[Z]All characteristics from normal flowers only.

[Y]Mean separation within columns by Duncan's multiple range test, 5% level.

[X]LDF-550, PSD-599, and open controls yielded too few normal flowers for proper replication.

[W]Data are means (± 1 SD).

This study has demonstrated the application of a simple prediction method as an aid in the choice of a polymeric film for MA packaging. The method determined a range of required film permeation for each unit of a commodity to be packaged to yield a chosen atmosphere. While a very specific package was developed and tested, further package parameter changes could be made, provided that the permeation to commodity ratio is maintained. The sealed package of LDF-301 film developed here maintained flowering ability of precooled tulip bulbs. Actual marketing studies with this package will be performed in the future. It is hoped that this method will aid others in modified atmosphere package development.

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LITERATURE CITED

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SECTION II

CONTROL OF INFECTION BY *PENICILLIUM* SPP. OF PRECOOLED TULIP BULBS IN A MODIFIED ATMOSPHERE PACKAGE

Abstract. Root plates of precooled tulip bulbs (Tulipa gesneriana L. 'Kees Nelis') maintained in sealed modified atmosphere packages of low density polyethylene film for 4 wks became infected with species of Penicillium. Infection led to increased ethylene and CO_2 as well as reduced O_2 levels in the packages. Infection also led to reduced rooting and increased floral abortion during subsequent forcing of the bulbs. Prochloraz and vanguard pretreatment of the bulbs prior to packaging controlled the damage caused by Penicillium spp. through 4 wks of storage in the packages. Control of infection resulted in equilibrium levels of ca. 5% O_2, 4% CO_2, and 0.1 µl/liter ethylene in the packages. Pretreated bulbs also retained excellent bulb flowering ability through 4 wks of storage while non-packaged bulbs flowered poorly. Benomyl, captan, and chlorine dip pretreatments did not control root plate infection in the packages.

The design and initial testing of a modified atmosphere (MA) package for marketing of precooled tulip bulbs has been outlined (Section I). This system, which consists of a consumer-size package of 5 bulbs sealed in a low density polyethylene film (LDF-301, Dow Chemical), was partially successful in maintaining flowering ability of the bulbs through 3 wks at 20°C. However, infection of the bulb

root plates by Penicillium spp. during storage appeared responsible for unfavorable package atmospheres and for the prevention of normal flowering upon forcing of the bulbs in the greenhouse. These effects of infection by Penicillium spp. could limit the future commercial success of the package.

MA packaging has been shown to increase the shelflife of various commodities by reducing transpirational water loss, product respiration, and the detrimental effects of ethylene exposure (3, 11, 14, 15). However, the high relative humidities in MA packages have been shown to create excellent environments for commodity infection by both fungi and bacteria. It appears that the common controlled atmosphere (CA) conditions of 2-3% O_2 and 5% CO_2 suppress only moderately most commodity disease organisms (4). Disease causing fungal organisms were observed to be detrimental to cut tulips (10, 20), carnations, and chrysanthemums (5) stored in various film packages, while the bacteria Erwinia carotovora caused decay of bell peppers packaged in polyethylene (PE) and polyvinylchloride (PVC) films (2). Anthracnose was seen to invade the stem end of avocados in individual PE bags after 40-50 days at 10°, even though the fruit were benomyl dipped prior to packaging (11). Various bacteria and fungi were isolated from flowering pot plants and bedding plants sealed in coextruded PE-polypropylene (PP) film packages (6). Although identification and pathogenicity studies of the isolates were not performed, decayed flowers and foliage made the plants unmarketable.

Fungicide or sterilant pretreatment of commodities has been instrumental to the success of some sealed packages. A chlorine dip controlled mold development on tomatoes in PVC and PE packages (14) which led to a

fruit shelflife of 21 days at 25°. Benomyl or thiabendazole controlled occasional infections by Gloeosporium musarum in bananas in large sealed PE shipping containers (15, 16). This MA packaging technique is now used commercially for distant market shipment of bananas (21). Thus, while disease growth is common in sealed commodity packages, fungicide pretreatment has led to control in some instances.

The control of infection by Penicillium spp. of precooled tulip bulbs in an MA package could make the marketing of these bulbs a commercial reality. Therefore, the purpose of this study was to determine the effectiveness of various fungicide pretreatments in controlling Penicillium root plate infection of the packaged bulbs. The nature of the detrimental effects of Penicillium growth upon both the package gaseous atmosphere and the subsequent flowering ability of the bulbs also was investigated.

Materials and Methods

This research was performed during the 1981-82 and 1982-83 bulb forcing seasons. Tulip bulbs (12-14 cm in circum.) were shipped to East Lansing, Michigan, from the Netherlands in open tray cases. Temperatures during shipment were 13-17° (1981-82) and 17-20° for 10 days followed by 4 days at 7-15° (1982-83). The shipping/arrival dates were Sept. 11/Oct. 6, 1981; and Aug. 16/30, 1982. All bulbs had reached stage G upon arrival and were stored at 13° (1981-82) and 17-20° (1982-83) until the 5° precooling period began.

Expt. 1. (1981-82). 'Kees Nelis' bulbs were precooled at 5° and 80-90% RH from Oct. 8 to Dec. 30 (12 wks). At the end of the precooling period, the bulbs were treated with various fungicides

prior to packaging in LDF-301 film. The following fungicides were applied: benomyl (Benlate 50 WP) [methyl-1-(butylcarbamoyl) benzimidazole-2 yl carbamate]; vanguard (CGA-64251 10 WG) [1-[[2-(2,4-dichlorophenyl)-4-ethyl, 1,3-dioxolan-2-yl]methyl]-1 H-1,2,4-triazole]; prochloraz (BTS-40 542 40 EC) [1-(N-propyl-N-(2-(2,4,6-trichlorophenoxy) ethyl)carbamoyl)imidazole]; and captan 50 WP [N-trichloromethyl-mercapto-4-cyclohexene-1,2-dicarboximide]. All fungicides were applied by dipping the bulbs for 20 min in well-agitated water suspensions in 4 liter containers at 21°. Captan was applied both as a suspension and as a dust. Two rates of each fungicide were used. These are shown in Table 1 in μg of active ingredient per ml. The low rate of captan dust was prepared from captan 50 WP and talc. Bulbs dipped in water without fungicide and non-dipped bulbs were utilized as controls. All dipped bulbs were thoroughly dried under flowing air from a small fan before packaging.

Five bulbs from each treatment then were sealed in each LDF-301 film package and placed randomly in a 20° room (40-50% RH) for durations of 2 and 3 wks. Two cm lengths of adhesive tape (Scotch® Patch and Repair Tape) were utilized as gas sampling ports on the film surface of the 3 wk packages. The top length of tape was used to seal previous sampling holes on the bottom length. At the end of each storage period the bulbs were removed from the packages, and the bulb tunics were removed. The percentage of the surface area of each bulb root plate infected with Penicillium spp. was estimated and recorded.

The package parameters and atmosphere monitoring, the use of initial post precooled controls, and the forcing of the bulbs were identical to those reported earlier (Section I), except that each pot

of 5 bulbs from every treatment was drenched with 0.2% benomyl and 0.4% ethazol after planting.

Upon forcing, the percent normal flowers obtained from each pot was recorded. In addition, the abnormal plants were rated for floral and shoot abnormalities. The 0-4 point rating system utilized was: 0 - no shoot elongation above the bulb; 1 - some shoot elongation, but flower unemerged from leaves; 2 - tepals visible, but dried and yellow; 3 - tepals turgid, but no color development; and 4 - abnormal or misshapen tepals. The ratings from each abnormal plant were averaged to yield an abnormality rating for each pot. For the correlation analysis all plants were rated, with normal flowers additionally rated as 5 points. These averages for each pot were designated as floral ratings.

After flowering, all bulbs were removed from the pots and the soil was carefully washed from the roots. They were blotted dry and cut from the bulbs, and an average bulb fresh root weight was determined for each pot.

Expt. 2. (1982-83). 'Kees Nelis' bulbs were precooled at 5° and 80-90% RH from Sept. 17 to Dec. 10 (12 wks). Some Penicillium growth was observed on the bulb tunics during the precooling period. To avoid development of high inoculum levels, the bulbs were dusted with plain talc on Nov. 23 to lower the available surface moisture for pathogen growth while avoiding fungicide use. At the end of the precooling period, one half of the bulbs had inoculum applied to them in addition to that naturally present. Application was by dipping the base of the bulbs in a spore suspension for a few seconds. The remaining bulbs had no additional inoculum applied to them. The mixed spore suspension

of ca. 10^7 spores/ml was prepared from cultures of various isolates of Penicillium spp. These isolates were collected during the 1981-82 season from infected bulbs and were maintained in culture on potato-dextrose agar. A few drops of Tween-20 surfactant were added to the suspension to facilitate spore wetting. Investigations of the pathogenicity, fungicide resistance, and other properties of the individual Penicillium isolates are reported elsewhere (Section IV).

Fungicide or sterilant treatments used before bulb packaging were: vanguard (240 µg a.i./ml), prochloraz (600 µg a.i./ml), and bleach (6000 ppm available chlorine, pH 7.6). In addition, water-dipped and non-dipped control bulbs were packaged. Both inoculated and non-inoculated, non-packaged control bulbs were utilized and initial post precooled bulbs were planted at the start of the experiment. The inoculation/fungicide/storage duration treatment combinations were arranged in a 2 x 6 x 2 factorial with 4 replications (packages) of each combination in a completely randomized design.

The fungicide application, packaging, storage, disease evaluation, planting, forcing, and flowering evaluation were the same as utilized in Expt. 1 except for the following changes. Bulbs were stored for 3 and 4 wks. The planting mix was 50% muck peat, 25% perlite, and 25% vermiculite. An additional fertilization of $Ca(NO_3)_2$ at 2.4 g/liter was applied one week after the first fertilization. At flowering, the fresh root weight was not determined. Instead, a 0-4 root rating was recorded for each bulb. A photograph of this rating scale was maintained to assure repeatability (Figure 10). An average root rating then was calculated for each pot.

Statistical Analysis. Analysis of variance was performed where possible. Data transformations were used where noted in the tables and figures. When the data could not be transformed to meet the assumptions of the analysis of variance, the Kruskal-Wallis non-parametric statistic was used for testing overall treatment significance, while the Mann-Whitney statistic was used for more specific mean separation.

Results and Discussion

Expt. 1. (1981-82). The root plate of the precooled tulip bulbs provided an excellent site for Penicillium infection. This was likely due to the slight emergence of the root initials from the root plate during the precooling and storage in LDF-301 film packages. This emergence is visible on the non-infected root plates shown in Figure 1. Infection also was seen on any occasional wounds on the outer fleshy scale of the bulbs. Saturated relative humidity conditions in the package were indicated by the soft and watersoaked tunics of bulbs removed from the packages as compared to the dry tunics of the bulbs at the time of packaging. This condition was ideal for infection since both free water and nutrients that may leach from the tissue have been demonstrated to be necessary for Penicillium spore germination (7, 12).

The infection of root plates after bulb prepackaging treatments and storage in LDF-301 film packages is shown in Table 1. The water dip pretreatment apparently spread spores to the root plates yielding greater infection than on non-dipped bulbs. In addition, uptake of water through the root plate during the 20 min treatment may have led

Figure 1. Infection by Penicillium spp. of precooled 'Kees Nelis' tulip bulb root plates pretreated with benomyl at 2000 µg a.i./ml (top) compared to non-infected bulbs treated with vanguard at 240 µg a.i./ml (bottom). All were packaged in LDF-301 film for 3 wks at 20°C (1981-82).

Table 1. Infection by Penicillium spp. of tulip bulb root plates after prepackaging dips or dusts and subsequent storage for 2 or 3 wks at 20°C in LDF-301 film packages or non-packaged (1981-82).

Prepackaging treatment	Rate[y] (µg a.i./ml)	% of root plate infected[z] 2 wks	3 wks
H_2O	----	34	65
No dip	----	0	10
Benomyl	1000	47 ns	88
	2000	54 ns	97
Prochloraz	300	0	2
	600	0	1
Vanguard	120	0	0
	240	0	1
Captan	1200	18 ns	48 ns
	2400	10 ns	47 ns
Captan dust	10%	1	4
	50%	0	3
Non-packaged			
H_2O	----	1	2
No dip	----	0	1

[z]Means within columns different from H_2O dip controls at 5% level by Mann-Whitney nonparametric statistic, except those marked nonsignificant (ns).

[y]Response from two application rates was not significantly different for any fungicide.

to deep spore penetration and enhanced infection. Non-dipped bulbs in the packages displayed a range of 0-80% infection of each bulb root plate after 3 wks although the average infection was only 10%. This large variation likely reflected differences in natural inoculum on the bulbs and/or bulb susceptibility differences.

Benomyl treatment yielded more infection than that observed on water dipped bulbs (Table 1 and Figure 1). This was most likely due to uninhibited growth of a benomyl-resistant isolate of P. corymbiferum that was later obtained in pure culture from the bulbs (Section IV). Both prochloraz (19) and vanguard (17), two new unregistered chemicals, yielded excellent control of infection. These compounds, at the rates utilized here, have been shown to be active against benomyl resistant P. expansum isolates on stored apples (1). Apparently, they are active against the resistant P. corymbiferum as well. No phytotoxic effects from these two chemicals were visible on the bulbs after removal from the packages or upon forcing in the greenhouse.

Captan treatment was unsuccessful as a dip application, but appeared to control infection when applied as a dust. Decreased bulb surface moisture and the lack of an inoculum spreading during dipping were likely explanations. The dust was unsightly and considered unacceptable from a consumer viewpoint. However, these results indicate that enclosure of a packeted desiccant in the package could possibly control the infection without the use of a fungicide, if bulb desiccation did not occur. This possibility deserves investigation in the future.

The effects of prepackaging treatment on package O_2 and CO_2 levels are shown in Figures 2 and 3 respectively. The treatments that

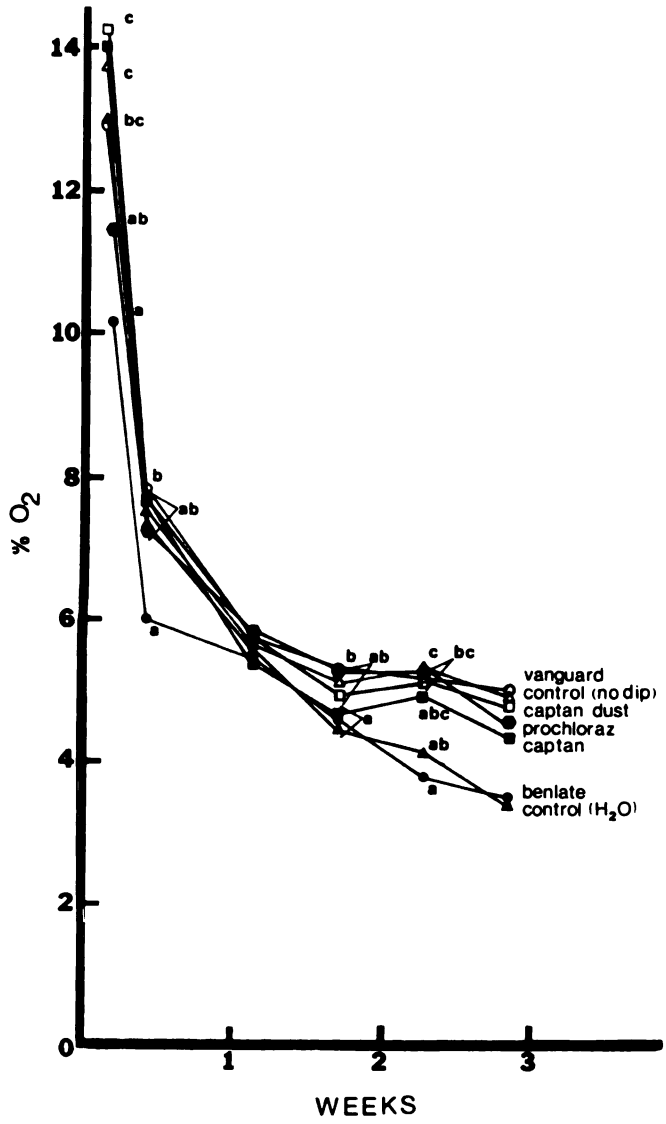

Figure 2. Effect of prepackaging treatment on O_2 levels in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C (1981-82). (Mean separation within sampling date by Duncan's multiple rante test, 5% level. Absence of letters indicates no significant differences on that date.)

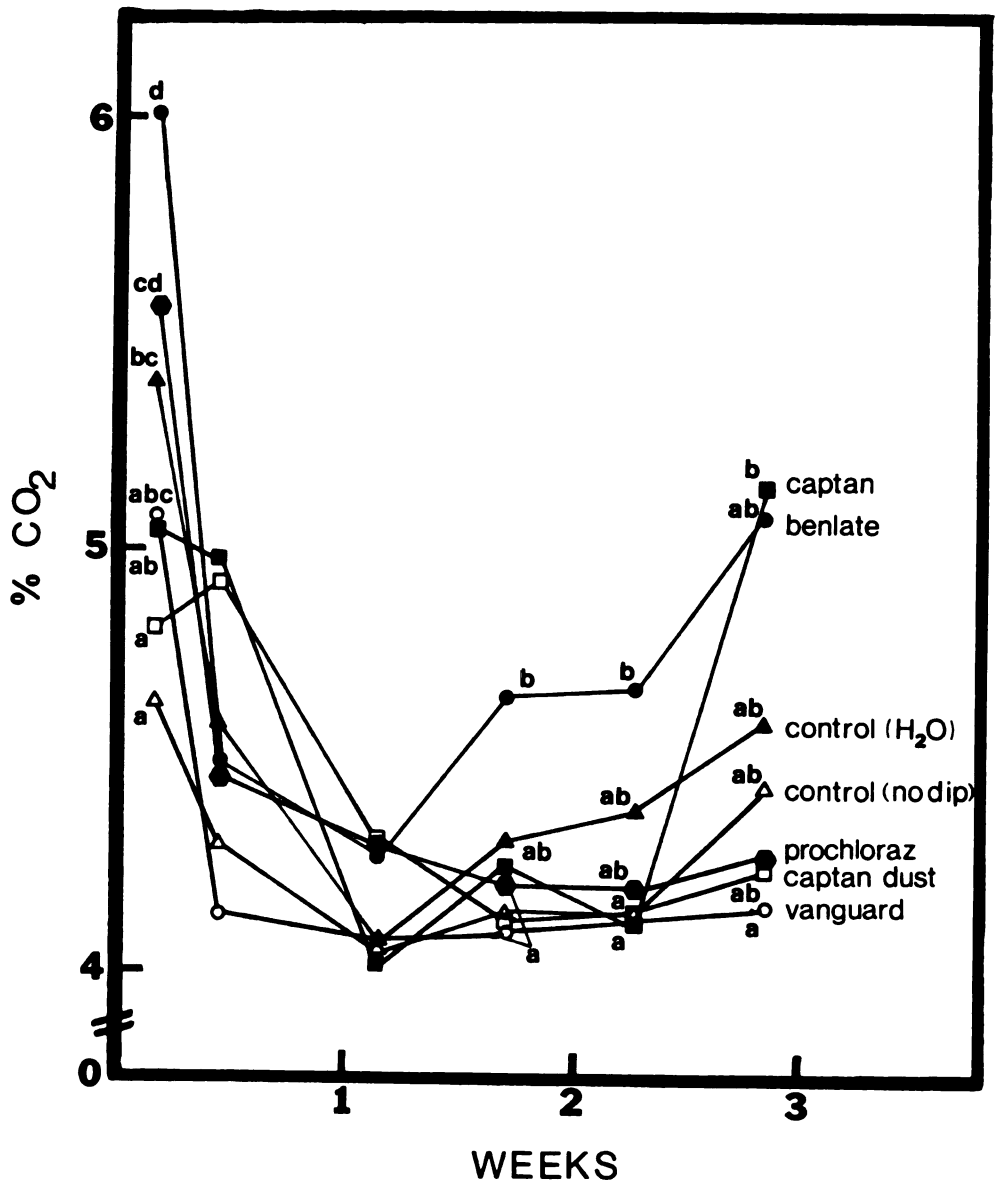

Figure 3. Effect of prepackaging treatment on CO_2 levels in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C (1981-82). (Mean separation within sampling date by Duncan's multiple range test, 5% level. Absence of letters indicates no significant differences on that date.)

yielded the most severe Penicillium infection (benomyl and water dips) resulted in the lowest package O_2 levels and the highest CO_2 levels. Ambient levels during storage were ca. 0.10% CO_2 and 20.2% O_2. Increased bulb respiration in response to infection and the additional respiration of the fungus itself were likely causes for the differences in package atmospheres. When Penicillium spp. were controlled, package equilibrium was obtained at ca. 5% O_2 and 4% CO_2. The packages of non-dipped bulbs did not show O_2 declines below 5% as was observed in earlier studies with bulbs that were not pretreated with fungicide (Section I). Those bulbs were packaged on Jan. 23 while bulbs for this study were packaged on Dec. 30. Bulb root plates have been observed to become more protruded as the period of storage after harvest but before planting lengthens (8). Greater root plate protrusion on bulbs utilized in the earlier trials could have increased susceptibility to infection and ultimately decreased the package O_2 levels. Differences in natural inoculum levels could have been involved also.

Package ethylene levels are shown in Figure 4. Tulip bulbs themselves have been demonstrated to produce ethylene in response to precooling (9, 13). The ethylene levels in packages of vanguard treated bulbs, where little Penicillium spp. growth occurred, were further evidence for this production. The subsequent forcing of the bulbs indicated that these ethylene levels in the package atmosphere were not detrimental to flowering. Increased package ethylene levels resulted from pretreatments that poorly controlled infection by Penicillium spp. Ambient levels during storage were ca. 50 nl/l. The benomyl resistant P. corymbiferum isolated from the bulbs has been shown to produce ethylene in pure culture. This isolate appeared

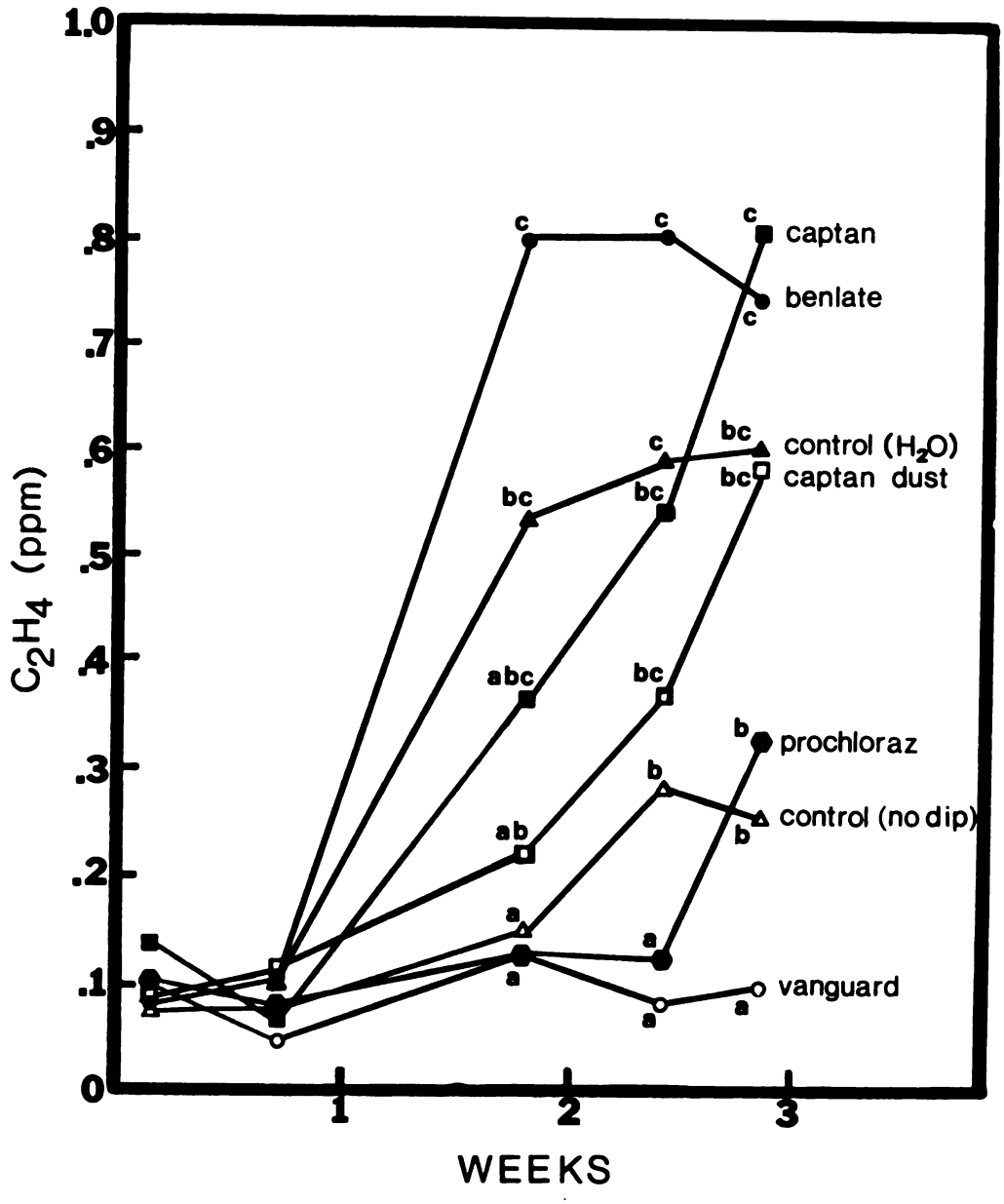

Figure 4. Effect of prepackaging treatment on ethylene levels in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C (1981-82). (Mean separation within sampling date by Duncan's multiple range test, 5% level. Absence of letters indicates no significant differences on that date. Data analyzed on log transformed scale.)

responsible for at least some of the ethylene accumulation, especially within packages of benomyl treated bulbs. However, increased bulb ethylene production in response to infection as well as pathogen ethylene production could have occurred in the packages (Section IV). The cause of the ethylene accumulation in packages of bulbs pretreated with captan dust was unclear.

The results of subsequent forcing of the bulbs after 2 and 3 wks of storage at 20° are shown in Tables 2 and 3 respectively. All treatments yielded nearly perfect flowering after 2 wks except for packages of benomyl and water dipped bulbs. The ratings indicated that the average abnormal flower from these two treatments had dried and yellow tepals. These two treatments also yielded the poorest root growth during forcing.

Prochloraz and vanguard dipped and captan dusted bulbs in packages yielded nearly perfect flowering after 3 wks of storage (Table 3). However, captan, water, and benomyl treated bulbs in packages flowered very poorly. The floral abnormalities and reduction of root growth observed during forcing of benomyl treated bulbs were even more severe after 3 wks than after 2 wks of storage. The non-pretreated packaged bulbs did flower acceptably after 3 wks. This indicated that natural inoculum and/or bulb susceptibility to infection in this experiment may have both been low enough to allow flowering without fungicide pre-treatment. Non-packaged control bulbs yielded only 40-60% normal flowers. Those non-packaged bulbs that were dipped in water had lower abnormality ratings and reduced root growth compared to their non-dipped counterparts. While little disease was evident on any non-packaged bulbs at the end of storage, some infection may have

Table 2. Flowering of precooled 'Kees Nelis' tulip bulbs after prepackaging treatment and subsequent storage for 2 wks at 20°C in LDF-301 film packages or non-packaged (1981-82).[z]

Prepackaging treatment	Rate (µg a.i./ml)	% normal flowers[y]	Abnormality rating[x]	Root fresh wt. (gm)[w]
H_2O	----	65	2.5	2.8ab
No dip	----	95	---	7.1cde
Benomyl	1000	*75 ns	2.0	3.7abc
	2000	45 ns	2.3	1.9a
Prochloraz	300	90	3.5	6.9cde
	600	100	---	7.4cde
Vanguard	120	100	---	7.8de
	240	100	---	8.8e
Captan	1200	80	---	5.1a-d
	2400	100	---	5.5a-e
Captan dust	10%	95	---	6.7b-e
	50%	100	---	8.6d-e
Non-packaged				
H_2O	----	100	---	6.1a-e
No dip	----	95	---	6.3b-e

[z]Initial post precooled controls yielded 100% normal flowers and 7.4 gm fresh root wt.

[y]Means different from H_2O dip controls at 5% level by Mann-Whitney nonparametric statistic except those marked nonsignificant (ns). Difference between fungicide rates at 5% level indicated by (*).

[x]No significant differences. Ratings shown only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated from 0-4 according to symptoms of shoot and floral abnormality (see text).

[w]Mean separation within columns by Duncan's multiple range test, 5% level.

Table 3. Flowering of precooled 'Kees Nelis' tulip bulbs after prepackaging treatment and subsequent storage for 3 wks at 20°C in LDF-301 film packages or non-packaged (1981-82).[z]

Prepackaging treatment	Rate (µg a.i./ml)	% normal flowers[y]	Abnormality rating[x,v]	Root fresh wt.(gm)[w,v]
H_2O	----	15	1.8cd	0.4abc
No dip	----	87	---	3.3efg
Benomyl	1000	5 ns	1.1abc	0.1ab
	2000	5 ns	0.4a	0.1a
Prochloraz	300	95	---	3.8fg
	600	95	---	3.8fg
Vanguard	120	100	---	5.0g
	240	100	---	4.2fg
Captan	1200	50	1.7bcd	1.5cde
	2400	45	2.5de	1.4b-e
Captan dust	10%	90	3.0e	4.4fg
	50%	95	---	4.6fg
Non-packaged				
H_2O	----	40	0.8ab	0.9a-d
No dip	----	60	2.3de	2.3def

[z]Initial post precooled controls yielded 100% normal flowers and 7.4 gm fresh root wt.

[y]Means different from H_2O dip controls at 5% level by Mann-Whitney nonparametric statistic except those marked nonsignificant (ns). There were no differences between fungicide rates.

[x]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated from 0-4 according to symptoms of shoot and floral abnormality (see text).

[w]Data were analyzed on log (X + 1) transformed scale.

[v]Mean separation within columns by Duncan's multiple range test, 5% level.

developed during forcing yielding the more severe symptoms of the dipped bulbs.

A negative correlation was evident between infection of the bulb root plate by Penicillium spp. and subsequent root growth after 2 and 3 wks of storage in packages (Figures 5 and 6). The root growth also was positively correlated with the floral rating. This indicated that floral abortion was possibly induced by a reduction in root growth during forcing. This reduction likely resulted from infection of the root plates in the packages. The increased package ethylene levels which resulted from infection also could have induced floral abortion.

Expt. 2. (1982-83). In spite of the application of spore suspension to the bulb bases, disease severity was similar to that on bulbs with only naturally occurring inoculum. Only prepackaging treatment and duration of storage led to significant differences in disease severity for this experiment (Table 4). It was unlikely that poor contact of the spores with the bulb root plate occurred during inoculation. The tunics of the bulbs were cracked near the protruding root plates, which allowed suspension contact with the root plates to be easily achieved. However, bulbs dipped in water and stored for 3 wks displayed less severe infection than similarly treated bulbs during 1981-82 (Table 1). This suggested that bulbs used in Expt. 2 were less susceptible to infection. Possibly infection sites on the root plate, rather than inoculum supply, were limiting to infection by Penicillium spp. The lack of increased disease incidence following

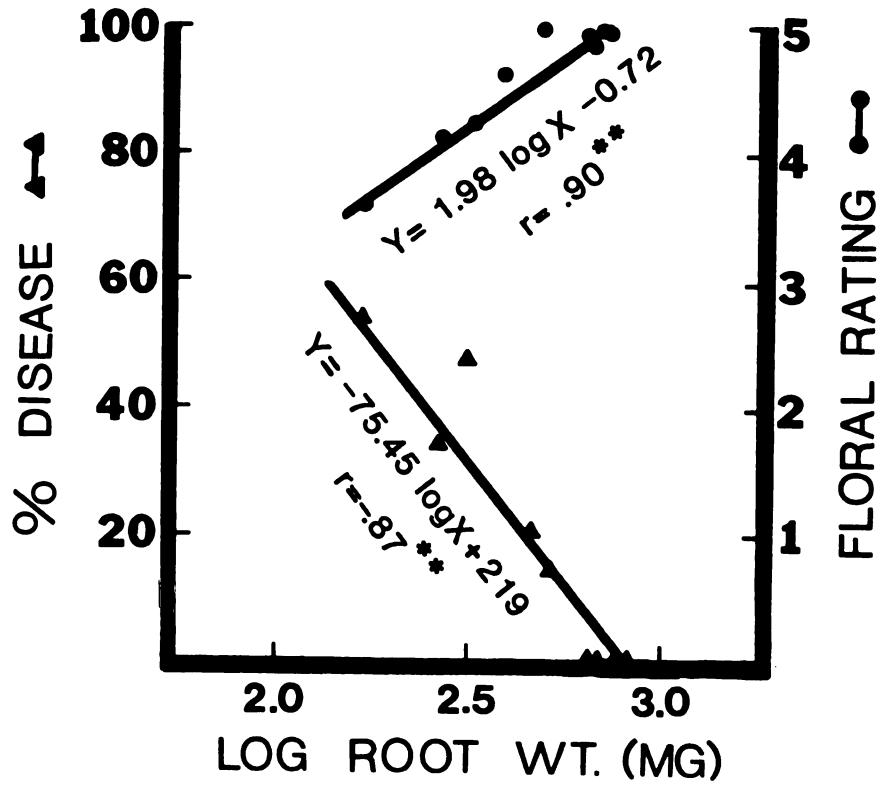

Figure 5. Correlations of fresh root weight with percentage of root plate diseased and floral ratings of precooled 'Kees Nelis' tulip bulbs after prepackaging treatments and subsequent storage for 2 wks at 20°C in LDF-301 film packages (1981-82). (Plotted are the means of 4 replications.)

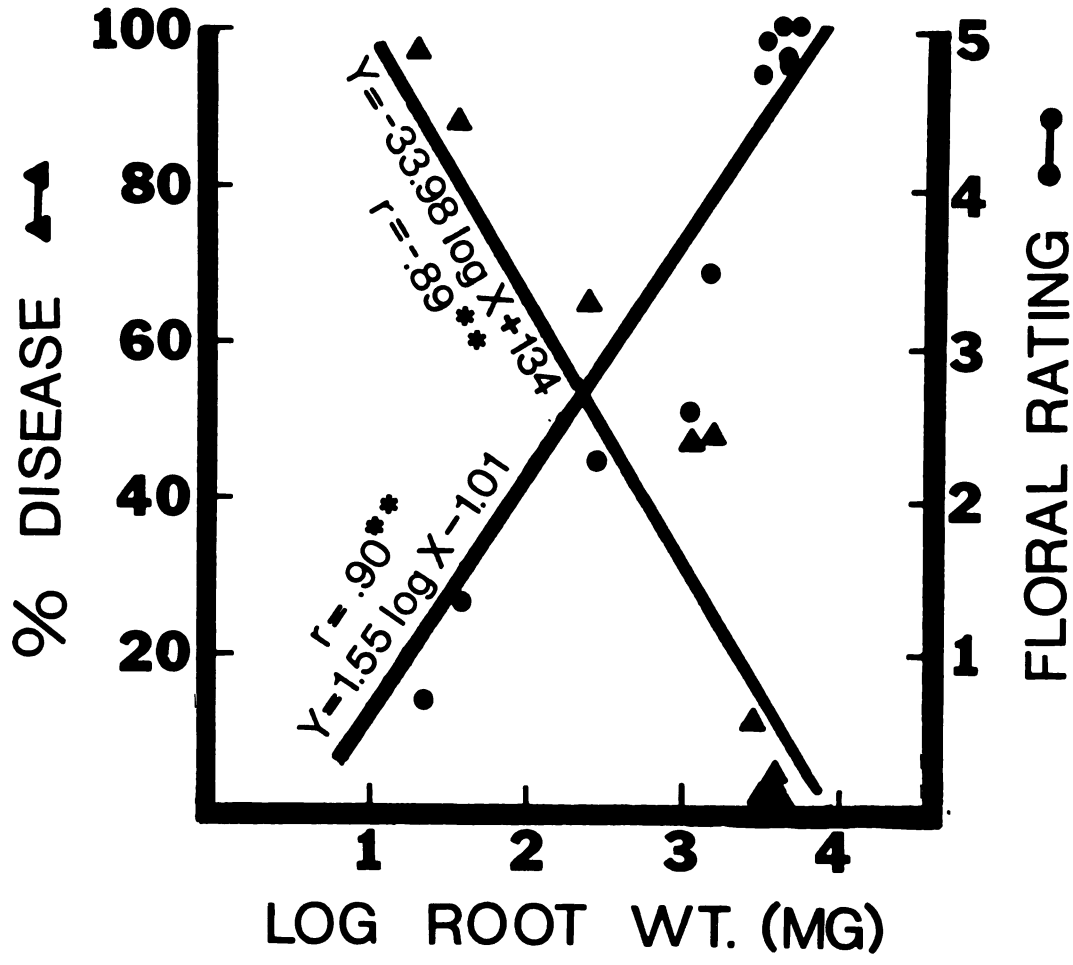

Figure 6. Correlations of fresh root weight with percentage of root plate diseased and floral ratings of precooled 'Kees Nelis' tulip bulbs after prepackaging treatments and subsequent storage for 3 wks at 20°C in LDF-301 film packages (1981-82). (Plotted are the means of 4 replications.)

Table 4. Infection by Penicillium spp. of precooled 'Kees Nelis' tulip bulbs after fungicide or sterilant prepackaging treatments and subsequent storage for 3 and 4 wks at 20°C in LDF-301 film packages or non-packaged (1982-83).[z]

Prepackaging treatment	% of root plate infected[y]	
	3 wks	4 wks
H_2O	26de	36ef
No dip	9c	26de
Chlorine (6000 ppm)	12cd	53f
Vanguard (240 µg a.i./ml)	0a	1ab
Prochloraz (600 µg a.i./ml)	2ab	3b
Non-packaged	1ab	3b

[z]Treatments applied to inoculated and non-inoculated bulbs. Inoculated bulbs dipped briefly in mixed spore suspension (10^7 spores/ml). Non-inoculated bulbs infected only by naturally-occurring inoculum. There were no significant infection level differences within any treatment between the inoculated and non-inoculated bulbs. Data shown are means of inoculated and non-inoculated bulbs.

[y]Data were analyzed on log (X + 1) transformed scale. Treatment x wks interaction significant at 5% level. Separation of any means by Duncan's multiple range test, 5% level.

application of inoculum in addition to that present on the bulbs was evidence for infection site limitations.

Vanguard and prochloraz again were most effective in controlling infection through 4 wks of storage (Table 4). Chlorine pretreatment was not successful. Apparently, not all spores were killed by the sterilant. Similar control failure has been observed with citrus inoculated with Penicillium and treated with chlorine (18). Van der Plank (18) suggested that oxidizable substances in the fruit peel reduced the active chlorine before it could penetrate to the pathogen deep within a wound. This may have occurred on the bulb root plates. Possibly, the solution did not even contact spores deeply imbedded in the root plates. Additionally, the lowering of the population of competetive microorganisms on the surface of the root plate may have ultimately increased the spread of infection during the duration of storage.

Package O_2 levels were again reduced when Penicillium infection was poorly controlled (Figure 7). However, little difference in package CO_2 levels was observed (Figure 8). Package ethylene levels also paralleled disease infection although the levels were ca. 50% of those seen in Expt. 1 (Figure 9). This could have been due to less severe infection, to less production by the bulbs, or to a different population mix of ethylene and non-ethylene producing Penicillium spp. on the root plates. Ambient storage conditions were ca. 0.22% CO_2, 20.6% O_2, and 20 nl/l ethylene.

Non-inoculated bulbs pretreated with vanguard or prochloraz before packaging yielded excellent flowering after 3 wks (Table 5) and 4 wks of storage (Table 6). While inoculation previously was

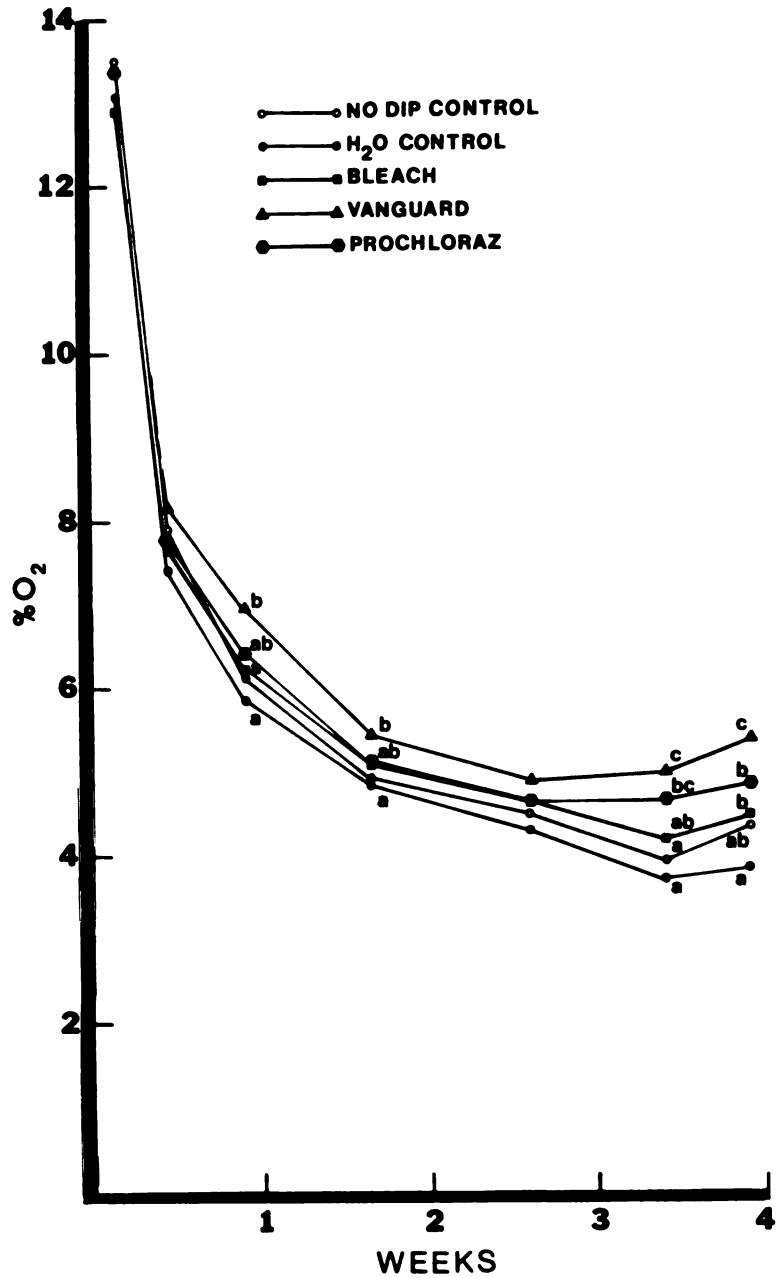

Figure 7. Effect of prepackaging treatment on O_2 levels in LDF-301 film packages of inoculated and non-inoculated precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C (1982-83). (Inoculation effect was nonsignificant. Data are means from packages of inoculated and non-inoculated bulbs. Mean separation within sampling date by Duncan's multiple range test, 5% level. Absence of letters indicates no significant differences on that date.)

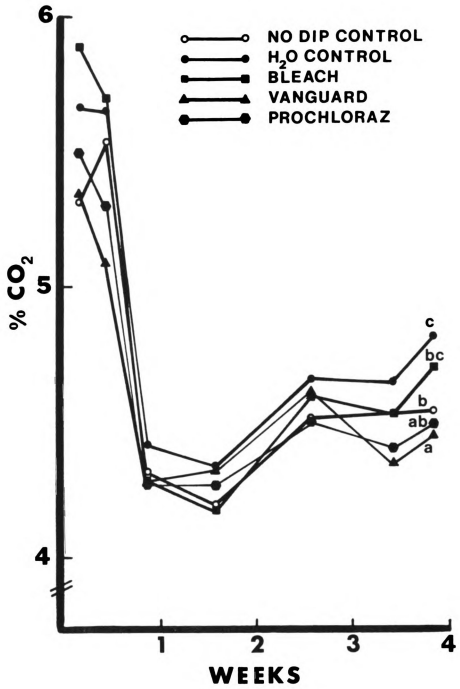

Figure 8. Effect of prepackaging treatment on CO_2 levels in LDF-301 film packages of inoculated and non-inoculated precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C (1982-83). (Inoculation effect was nonsignificant. Data are means from packages of inoculated and non-inoculated bulbs. Mean separation within sampling date by Duncan's multiple range test, 5% level. Absence of letters indicates no significant differences on that date.)

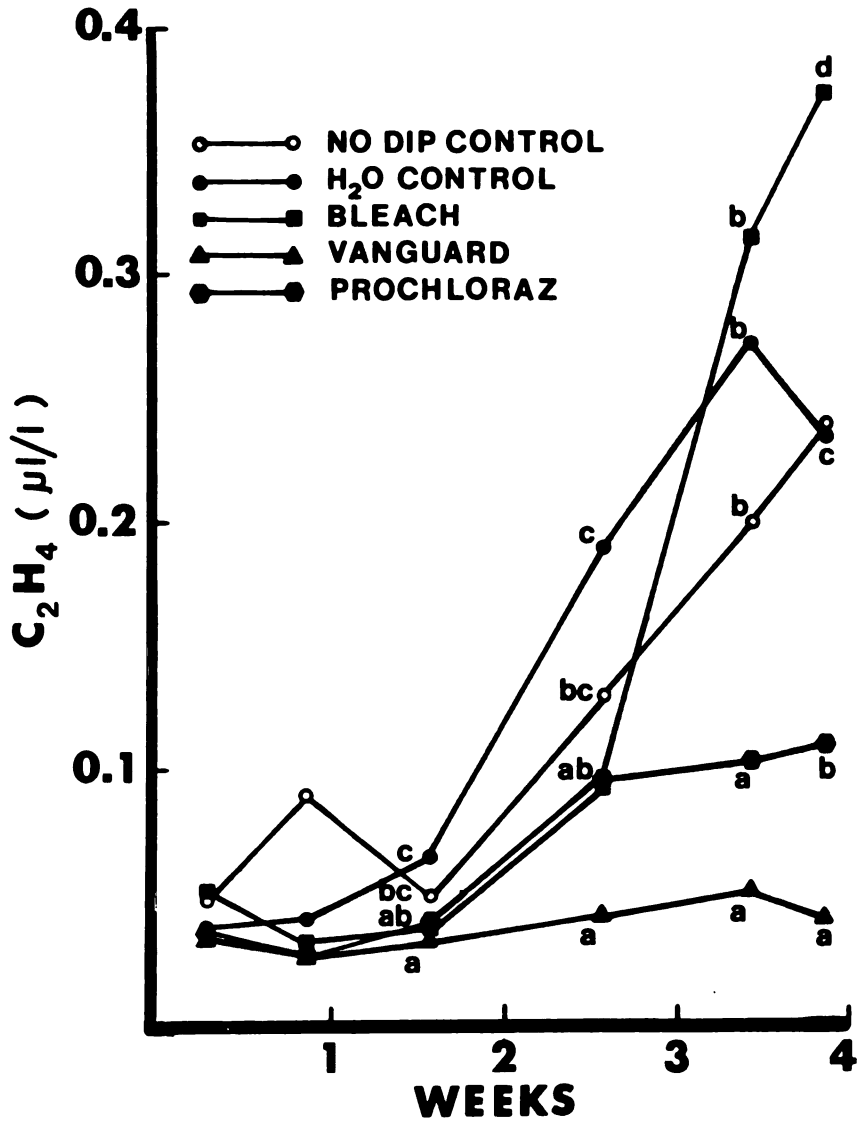

Figure 9. Effect of prepackaging treatment on ethylene levels in LDF-301 packages of inoculated and non-inoculated precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C (1982-83). (Data were analyzed on log transformed scale. Inoculation effect was non-significant. Data are means from packages of inoculated and non-inoculated bulbs. Mean separation within sampling date by Duncan's multiple range test, 5% level. Absence of letters indicates no significant differences on that date.)

Table 5. Flowering of inoculated and non-inoculated precooled 'Kees Nelis' tulip bulbs after fungicide or sterilant prepackaging treatments and subsequent storage for 3 wks at 20°C in LDF-301 film packages or non-packaged (1982-83).[z]

Prepackaging treatment	Inoculation (±)	% normal flowers[y]	Abnormality rating[x]	Root rating[w]
H_2O	-	80	2.8	3.4abc
	+	70	2.1	3.0a
No dip	-	55	3.0	3.5abc
	+	60	2.9	3.7bc
Chlorine	-	85	3.0	3.1a
	+	65	3.1	3.3ab
Vanguard	-	100*	---	3.9c
	+	* 60	3.2	3.9c
Prochloraz	-	80	3.0	3.8bc
	+	95	---	3.8bc
Non-packaged	-	60	3.1	3.9c
	+	60	2.9	3.8bc

[z]Initial post precooled control bulbs yeilded 95% normal flowers and a root rating of 3.9.

[y](*) to the right of means within column indicates difference at 5% level from H_2O by Mann-Whitney nonparametric statistic. (*) between means indicates significant inoculation effect by same statistic.

[x]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated from 0-4 according to symptoms of shoot and floral abnormality (see text). There were no significant differences.

[w]Root growth from each bulb rated from 0-4 (see Figure 10). Mean separation by Duncan's multiple range test, 5% level.

Table 6. Flowering of inoculated and non-inoculated precooled 'Kees Nelis' tulip bulbs after fungicide or sterilant prepackaging treatments and subsequent storage for 4 wks at 20°C in LDF-301 film packages or non-packaged (1982-83).[z]

Prepackaging treatment	Inoculation (±)	% normal flowers[y]	Abnormality rating[x]	Root rating[w]
H_2O	-	75	1.2	2.9bc
	+	70	2.3	3.0c
No dip	-	80	3.1	2.9bc
	+	90	1.5	3.4cd
Chlorine	-	40*	1.7	1.6a
	+	45	1.9	1.9ab
Vanguard	-	95*	---	3.9d
	+	95*	---	3.9d
Prochloraz	-	100*	---	3.8d
	+	100*	---	3.8d
Non-packaged	-	5*	1.2	3.7d
	+	0*	1.7	3.7d

[z]Initial post precooled control bulbs yielded 95% normal flowers and a root rating of 3.9.

[y](*) to the right of means within column indicates difference at 5% level from H_2O by Mann-Whitney nonparametric statistic.

[x]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated from 0-4 according to symptoms of shoot and floral abnormality (see text). There were no significant differences.

[w]Root growth from each bulb rated from 0-4 (see Figure 10). Mean separation by Duncan's multiple range test, 5% level.

shown not to increase disease in the package during 3 wks of storage, it did yield reduced flowering with vanguard treated bulbs. Even though good root development was obtained from these inoculated bulbs, the roots did exhibit a brown discoloration which may have indicated disease development. This discoloration was similar to that seen during pathogenicity trials with inoculated excised root plates (Section IV). While a benomyl and ethazol post planting drench was used as a standard treatment for prevention of Pythium and Rhizoctonia root rots, it appeared not to prevent this effect. When the roots emerged from the bulbs after planting, they may have been exposed to a large spore population remaining on the bulb surface that induced the discoloration. Neither this effect nor reduction of flowering from inoculated vanguard treated bulbs was seen after 4 wks of storage. Possibly some greenhouse environmental factor during the first week of forcing enhanced disease development on the 3 wk bulbs. Additional vanguard application at planting or an increased pretreatment application rate may prevent this occurrence.

Poor flowering and poor root growth resulted with chlorine pretreated bulbs after 4 wks of storage due to poor control of infection by Penicillium spp. in the packages (Table 6). The benefit of the LDF-301 film package was highly apparent after 4 wks of storage, with vanguard and prochloraz pretreated bulbs yielding nearly perfect flowering while non-packaged bulbs flowered negligibly (Table 6 and Figure 10). However, good root growth was obtained from the non-packaged bulbs.

In conclusion, infection of the root plate of precooled tulip bulbs by Penicillium spp. has been shown to be detrimental to the

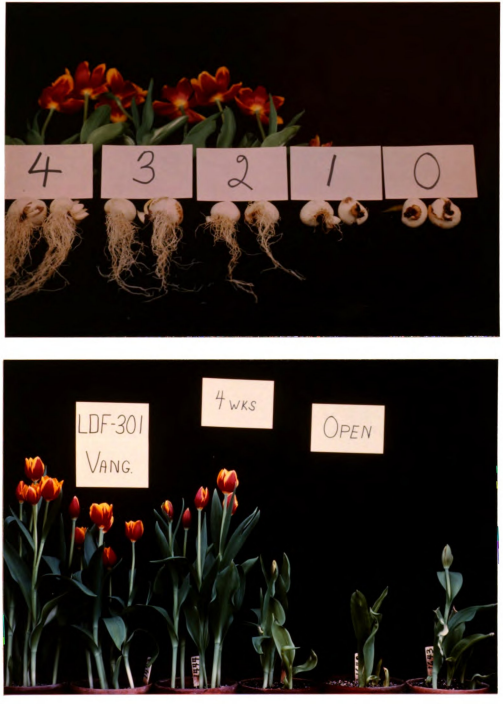

Figure 10. Representation of 0-4 root rating (top). Flowering of non-inoculated 'Kees Nelis' tulip bulbs (bottom) after vanguard pretreatment and 4 wks of storage at 20°C in LDF-301 film packages (left 3 pots) or non-packaged (right 3 pots).

function of the LDF-301 film packages. Infection increased CO_2 and ethylene levels and reduced O_2 levels in the packages. Decreased root growth and increased floral abortion during forcing were the end results of infection. Moderate amounts of infection by Penicillium spp. occurred on non-pretreated packaged bulbs in these studies. However, temperature fluctuation under actual marketing conditions could lead to condensation in the packages (Section III) which could increase fungus growth. Variability in natural inoculum levels and/or bulb susceptibility to infection also could exist. Therefore, fungicide pretreatment seems warranted. Benomyl pretreatment was unsuccessful due to the presence of a tolerant isolate of P. corymbiferum on the bulbs. However, prochloraz and vanguard, two unregistered fungicides, controlled infection in the packages.

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SECTION III

CULTIVAR RESPONSE, TEMPERATURE FLUCTUATION EFFECTS AND BULB ORGAN FRESH AND DRY MATTER DISTRIBUTION IN A MODIFIED ATMOSPHERE PACKAGE OF PRECOOLED TULIP BULBS

Abstract. 'Abra', 'Bing Crosby', 'Favourite', and 'Parade' tulip bulbs (Tulipa gesneriana L.) yielded 87-93% normal flowers after 3 wks in low density polyethylene film packages at 20°C whereas non-packaged bulbs displayed aborted flowers. Storage of the same cultivars for 4 wks in the packages was less successful. Infection of some bulbs by Fusarium oxysporum caused package ethylene levels to rise to 2-47 µl/liter which reduced subsequent flowering. Similar packages of 'Kees Nelis' bulbs stored at 20° for 2 wks followed by 3 wks of temperature fluctuation between 15 and 25° displayed little change in package CO_2 and O_2 levels. The temperature adaptability appeared due to both changing bulb respiration rates and changing film permeabilities to CO_2 and O_2. Packaged bulbs at any of the temperatures used yielded 80-100% normal flowers after 3 total storage wks with slightly reduced flowering after 4 wks. Non-packaged 'Kees Nelis' bulbs at 20° and 40-50% RH lost 30% of bulb fresh weight (FW) and 25% of bulb dry weight (DW) during 4 wks of storage. The scales of these bulbs lost 45% of FW and 35% of DW; the floral shoot lost 38% of FW and 20% of DW; while the root plate displayed a 37% loss in tissue hydration level. The daughter bulbs within these non-packaged bulbs displayed 7-fold increases in FW and DW during 4 wks. Bulbs in packages yielded little change in FW or DW of any bulb organs.

Control of infection of precooled tulip bulbs by Penicillium spp. in a modified atmosphere (MA) package has led to maintenance of bulb flowering ability for as long as 4 wks at a constant 20°C (Section II). However, temperature fluctuation can be expected under marketing conditions. Any resultant change in the package atmosphere must not be detrimental to subsequent flowering of the bulbs if success of the package is to be achieved in the marketplace.

There is conflicting information in the literature on temperature fluctuation effects upon sealed packages. Tomkins (27) reported that CO_2 increased with temperature in sealed packages of various commodities. He also observed that condensation could occur in response to temperature decline, leading to increased mold infestation. Consumer-size polyvinylchloride (PVC) packages have been reported to extend the shelflife of bananas at 15°, while odor and abnormal taste development was apparent at 22° (4). Roses packaged in various films kept well for 5 days at 0-10°, but not at higher temperatures (12). In his review of packaging, Hardenburg (11) displayed data from packages of green beans and the effects of temperature upon the internal package atmosphere. He concluded that temperature had profound effects upon package CO_2 and O_2 levels yielding little hope for practical MA packages. However, a reexamination of his data suggests that his packages were not properly optimized so that all eventually would have become anaerobic. Apparent in his data is the effect of temperature upon the rate of O_2 decline and not upon an ultimate equilibrium level.

No great atmospheric differences were found between individually sealed polyethylene bags of avocados at 20 or 30° (3). Henig and

Gilbert (13) found that final CO_2 and O_2 levels were nearly the same at 15° and 23° for PVC packages of tomatoes. They suggested that temperature changes affected both respiratory activity and film permeability to the same degree with their packages. Karel (16) demonstrated the temperature dependence of film permeability and found the level of dependence to vary with film type. The possibility that changes in film permeability to CO_2 and O_2 could allow an MA package of precooled tulip bulbs to adapt to a reasonable marketplace temperature fluctuation was investigated in this study.

Prince and coworkers (22) have demonstrated that storage of precooled bulbs in a 3-5% O_2 atmosphere leads to reduction in both bulb respiration rate and floral abortion due to ethylene exposure. These reductions may allow the maintenance of bulb flowering ability in the MA package. Ethylene induced shoot abortion is apparently prevented while the reduced respiration maintains high bulb carbohydrate levels for subsequent shoot elongation. However, LeNard (18) has recently suggested that when the planting or rooting of tulip bulbs is delayed after the precooling period, it is the predominance of daughter bulb enlargement that leads to floral shoot abortion. These studies report the effects of MA packaging upon daughter bulb enlargement and other bulb organ changes that occur during 20° storage. The varied response of 12 tulip cultivars to the MA packaging also is reported.

Materials and Methods

The study of fresh and dry matter distribution among the bulb organs was performed during the 1981-82 forcing season while the

cultivar evaluation and temperature fluctuation studies were performed during the 1982-83 season. Tulip bulbs (12-14 cm in circum.) were shipped to East Lansing, Michigan, from the Netherlands in open tray cases. Temperatures during shipment were 13-17° (1981-82) and 17-20° for 10 days followed by 4 days at 7-15° (1982-83). The shipping/arrival dates were Sept. 11/Oct. 6, 1981, and Aug. 16/30, 1982. All bulbs had reached stage G upon arrival. They were stored at 13° (1981-82) or 17-20° (1982-83) prior to the precooling except for the bulbs utilized in the temperature study, which were moved to 13° on Oct. 20 for storage prior to precooling. All bulbs utilized in the 1982-83 season had talc applied on Nov. 23 to lower the available surface moisture to reduce growth of Penicillium spp. during the precooling. All bulbs from both seasons were dipped in vanguard solution (240 µg a.i./ml) at the end of the precooling period as previously described for control of Penicillium spp. (Section II).

Cultivar Evaluation. Bulbs of 12 cultivars were precooled at 5° and 80-90% RH from Sept. 20 to Dec. 22 (13 wks). Following the precooling and fungicide treatment, one half of the bulbs were sealed in LDF-301 film packages (5 bulbs/package) and the remainder were not packaged. Three packaged and non-packaged replicates of each cultivar were placed randomly in a 20° storage room (40-50% RH) for each 3 or 4 wk duration. All other materials and methods utilized were identical to those in Expt. 2 of Section II, except that no bulbs were inoculated and only 2 pots of each cultivar were planted as initial post precooled controls.

Upon forcing, the percent normal flowers obtained from each pot was recorded. In addition, the abnormal plants were rated for floral and shoot abnormalities. The 0-4 point rating system utilized was: 0 - no shoot elongation above the bulb; 1 - some shoot elongation, but flower unemerged from leaves; 2 - tepals visible, but dried and yellow; 3 - tepals turgid, but no color development; and 4 - abnormal or misshapen tepals. The ratings from each abnormal plant were averaged to yield an abnormality rating for each pot. At flowering, a 0-4 root rating was recorded for each bulb, with 0 indicating no root growth and 4 indicating excellent growth. A photograph of this rating scale is shown in Section II. An average root rating was then calculated for each pot.

Temperature Fluctuation Study. 'Kees Nelis' bulbs were precooled at 5° and 80-90% RH from Nov. 2, 1982, to Jan. 27, 1983 (12 wks). Following the precooling and fungicide treatment, one half of the bulbs were sealed in LDF-301 film packages (5 bulbs/package) and the remainder were not packaged. All were placed at 20° (40-50% RH) for 1 wk to allow package atmosphere equilibrium to be obtained. Four packaged and non-packaged replicates then were randomly assigned to each of 6 temperature regimes for an additional 1, 2, or 3 wks of storage. The temperature regimes utilized were: constant 20°; constant 25°; constant 15°; 25° for 2 days/20° for 5 days; 15° for 2 days/20° for 5 days; and 25° for 2 days/15° for 2 days/20° for 3 days. The fluctuating temperatures were repeated each week. The ambient RH was 20-25% at 25° and 25-35% at 15°. Four packaged and non-packaged replicates of each treatment were removed at the end of

2, 3, and 4 total storage wks for forcing evaluation. All other materials and methods including the floral and root ratings were as described above for the cultivar evaluation except that 4 pots of initial post precooled control bulbs were planted at the start of the experiment. The permeability of the LDF-301 film at 15, 20, and 25° was determined with a custom-made permeability cell as previously described (Section I).

Bulb Organ Study. 'Kees Nelis' bulbs were precooled at 5° and 80-90% RH from Dec. 7, 1981, to March 9, 1982 (13 wks). Following precooling and fungicide treatment, one half of the bulbs were sealed in LDF-301 film packages (5 bulbs/package) and the remainder were not packaged. All were randomly placed at 20° (40-50% RH). At the start of the experiment and at weekly intervals through 4 wks, 3 replicates of packaged and non-packaged bulbs were removed from storage. Two bulbs from each replicate were randomly selected for dissection into shoot, inner daughter bulbs, outer daughter bulb, scales, and root plate. The separate organs from the 2 bulbs were pooled and the fresh and dry matter were determined. The outer daughter bulb, which underlies the papery tunic, was analyzed separately from the other daughter bulbs due to previously-observed size variability and occasional absence due to bulb handling.

Statistical Analysis. The analysis of variance or the Mann-Whitney nonparametric statistic was used. Statistical comparison of forcing results between temperature regimes was not utilized since the temperature rooms were not actually replicated. However, comparison of forcing results between packaged and non-packaged bulbs

was valid. The validity of the statistical comparison between package atmospheres at different temperatures was limited due to the replication problem. Trend analysis was utilized for the bulb organ study to elucidate duration and treatment effects. Separate analyses were performed for the fresh weight, dry weight, and fresh/dry weight ratio trends for each bulb organ.

Results and Discussion

Cultivar Evaluation. The package CO_2 and O_2 levels during 4 wks of storage are shown in Figures 1 and 2, respectively, for 3 of the cultivars utilized. 'Prominence' bulbs had the lowest mass of the cultivars utilized; 'Abra' bulbs were intermediate; and 'Oskar' bulbs had the highest mass. The small differences in package atmospheres observed were likely due to the different masses of tissue respiring within the packages. The other cultivars all yielded package atmospheres within the same range. Thus it appears that different film surface areas need not be utilized for packaging different cultivars to achieve a desirable atmosphere.

'Abra', 'Bing Crosby', 'Favourite', and 'Parade' bulbs all flowered acceptably (87-93% normal flowers) after 3 wks in LDF-301 film packages at 20° (Table 1). The other cultivars did not flower acceptably. It appeared that some of the cultivars did not respond well to the precooling technique itself as indicated by the flowering of initial post precooled control bulbs. All non-packaged bulbs flowered poorly after 3 storage wks and had abnormalities that ranged from no shoot growth to dried and yellow tepals. Except for 'Apeldorn', 'Golden Oxford', and 'Oxford', the abnormalities displayed by the packaged bulbs were less severe than from non-packaged bulbs.

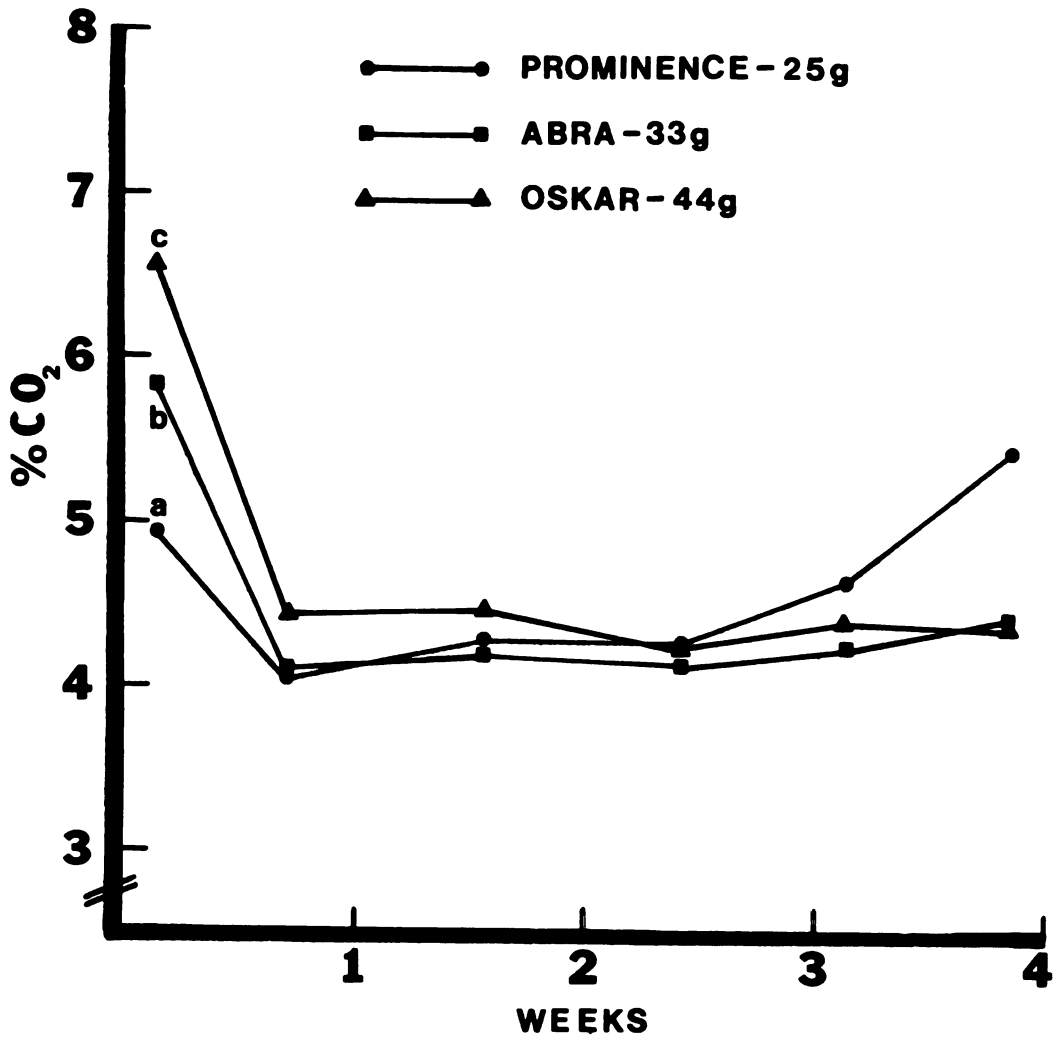


Figure 1. CO_2 levels in LDF-301 film packages of 'Prominence', 'Abra', and 'Oskar' precooled tulip bulbs through 4 wks at 20°C. (Mean separation within sampling date by Duncan's multiple range test, 5% level. No letters indicates no significant differences on that date. None of the other cultivars displayed significantly higher or lower values than the range shown on any date. Also shown are the average per bulb fresh weights for the 3 cultivars.)

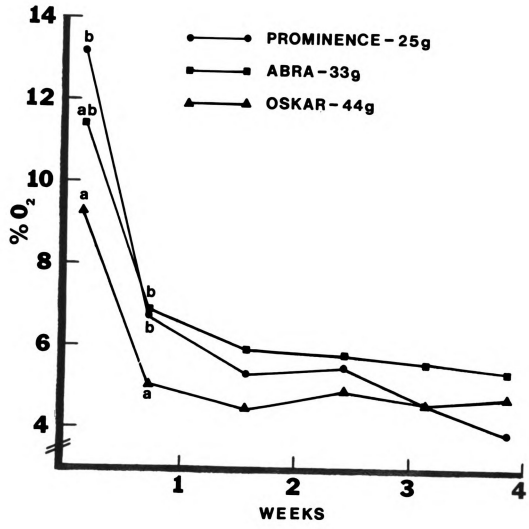


Figure 2. O_2 levels in LDF-301 film packages of 'Prominence', Abra', and 'Oskar' precooled tulip bulbs through 4 wks at 20°C. (Mean separation within sampling date by Duncan's multiple range test, 5% level. No letters indicates no significant differences on that date. None of the other cultivars displayed significantly higher or lower values than the range shown on any date. Also shown are the average per bulb fresh weights for the 3 cultivars.)

Table 1. Flowering of 12 cultivars of precooled tulip bulbs after storage for 3 wks at 20°C in LDF-301 film packages or non-packaged.[z]

Cultivar	% normal flowers[y]			Abnormality ratings[x,v]		Root ratings[w,v]	
	non-packaged		LDF 301	non-packaged	LDF 301	non-packaged	LDF 301
Abra	0	*	93	0.8a-e	--	1.9c-e	3.7gh
Apeldorn	0	*	73	1.6c-g	2.5g	2.9e-g	3.8gh
Bing Crosby	0	*	93	0.3ab	--	3.1f-h	3.9h
Favourite	13	*	87	0.5a-c	--	1.1a-c	3.9h
Golden Melody	0		27	0.0a	1.3c-f	0.2a	2.2d-f
Golden Oxford	7		60	2.0fg	2.0fg	3.7gh	3.9h
Monte Carlo	0		27	0.0a	1.7d-g	3.5ab	3.3ab
Oskar	0	*	47	0.6a-d	2.4fg	1.7bd	3.4gh
Oxford	7	*	67	1.9e-g	2.6g	3.7gh	3.9h
Parade	13	*	93	1.9e-g	--	3.5gh	4.0h
Paul Richter	0	*	47	0.3ab	2.4fg	0.6a	3.5gh
Prominence	0	*	67	0.1a	2.0fg	0.8ab	3.5gh

[z]Initial post precooled control bulbs yielded 100% normal flowers except Golden Oxford and Bing Crosby--80%; Oskar and Parade--70%; and Oxford and Monte Carlo--60%. All yielded root ratings of 3.6-4.0.

[y](*) indicates significant difference between packaged and non-packaged bulbs within cultivar at 5% level by Mann-Whitney nonparametric statistic.

[x]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated 0-4 according to symptoms of shoot and floral abnormality (see text).

[w]Root growth from each bulb rated 0-4 (see text).

[v]Mean separation between rows and columns by Duncan's multiple range test, 5% level.

The root ratings of packaged bulbs after 3 storage wks were in the 3.3-3.9 range for all cultivars except 'Golden Melody'. This suggested that rooting differences were likely not responsible for the flowering differences observed between packaged bulbs of the different cultivars. The root rating of non-packaged bulbs of 'Golden Oxford', 'Monte Carlo', 'Oxford', and 'Parade' were in the 3.5-3.7 range. This indicated that poor rooting of non-packaged bulbs was not the limiting factor for all cultivars but may have been for some of them after 3 wks of storage. None of the cultivars evaluated appeared to respond to the packaging as well as 'Kees Nelis', the cultivar utilized in other studies (Section II), since none of the tested cultivars flowered acceptably after packaging for 4 wks (Table 2). However, some did flower significantly better than their non-packaged counterparts. The floral abnormalities from packaged and non-packaged bulbs after 4 wks appeared more severe than after 3 wks of storage. Root growth was poor from most non-packaged cultivars. Rooting may have become an increasingly limiting factor for non-packaged bulbs between the 3 and 4 wk durations due to root plate desiccation (see below). 'Abra', 'Golden Oxford', 'Oxford', and 'Parade' packaged bulbs rooted well despite the poor flowering obtained.

Infection by Fusarium oxysporum f. sp. tulipae appeared to limit packaging success with many of the cultivars (Table 3). 'Abra', 'Apeldorn', 'Favourite', 'Golden Melody', 'Oskar', 'Paul Richter', and 'Prominence' bulbs all displayed some infection at the end of 4 wks of storage. Infection occurred at the root plate and was quite variable. Infection created extremely variable package ethylene levels, depending

Table 2. Flowering of 12 cultivars of precooled tulip bulbs after storage for 4 wks at 20°C in LDF-301 film packages or non-packaged.[z]

Cultivar	% normal flowers[y]		Abnormality ratings[x,v]		Root ratings[w,v]	
	non-packaged	LDF 301	non-packaged	LDF 301	non-packaged	LDF 301
Abra	0 *	33	0.3a-c	2.2e-g	1.2a-c	3.7gh
Apeldorn	0	0	1.0a-d	0.9a-d	1.5b-d	2.3c-e
Bing Crosby	0	20	0.3a-c	3.0g	1.9c-e	2.6d-g
Favourite	0	40	0.3a-c	0.9a-d	0.6ab	2.5d-f
Golden Melody	0	0	0.0a	0.2a-c	0.1a	0.6ab
Golden Oxford	0 *	47	0.3a-c	2.6g	1.1a-c	3.5f-h
Monte Carlo	0	0	0.0a	1.2de	2.6e-g	2.7e-g
Oskar	0	7	0.1ab	2.3fg	0.6ab	2.5d-f
Oxford	0 *	53	0.5a-d	3.0g	1.9c-e	3.7gh
Parade	0	7	1.4d-f	2.2e-g	3.0e-h	3.9h
Paul Richter	0	20	0.1ab	1.1b-d	0.1a	2.5d-f
Prominence	0	0	0.0a	0.9a-d	0.2a	2.1c-e

[z]Initial post precooled control bulbs yielded 100% normal flowers except Golden Oxford and Bing Crosby--80%; Oskar and Parade--70%; and Oxford and Monte Carlo--60%. All yielded root ratings of 3.6-4.0.

[y](*) indicates significant difference between packaged and non-packaged bulbs within cultivar at 5% level by Mann-Whitney nonparametric statistic.

[x]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated 0-4 according to symptoms of shoot and floral abnormality (see text).

[w]Root growth from each bulb rated 0-4 (see text).

[v]Mean separation between rows and columns by Duncan's multiple range test, 5% level.

Table 3. Ethylene levels after 5, 17, and 27 days, and *Fusarium oxysporum* presence, on 12 cultivars of tulip bulbs in LDF-301 film packages during 4 wks of storage at 20°C.

Cultivar	C_2H_4 Range (µl/liter)			*Fusarium*[z] presence
	Day 5	Day 17	Day 27	
Abra	0.05 - 0.08	0.33 - 0.81	0.10 - 2.06	+
Apeldorn	0.04 - 0.06	0.07 - 5.38	0.29 - 46.9	+
Bing Crosby	0.03 - 0.04	0.05 - 0.23	0.07 - 0.17	0
Favourite	0.06 - 0.15	0.35 - 0.68	0.89 - 14.3	+
Golden Melody	0.05 - 3.28	0.27 - 15.3	7.27 - 29.8	+
Golden Oxford	0.03 - 0.05	0.05 - 0.21	0.07 - 0.19	0
Monte Carlo	0.06 - 0.07	0.35 - 0.87	0.35 - 1.70	0
Oskar	0.03 - 0.16	0.16 - 1.29	0.17 - 8.00	+
Oxford	0.03 - 0.14	0.06 - 0.07	0.06 - 0.18	0
Parade	0.02 - 0.04	0.12 - 0.21	0.05 - 0.50	0
Paul Richter	0.15 - 0.21	0.24 - 10.2	6.28 - 37.3	+
Prominence	0.05 - 0.22	0.42 - 3.51	2.44 - 17.7	+

[z](0) indicates no *Fusarium* observed; (+) indicates *Fusarium* observed on 1 or more bulbs in at least 1 package.

upon whether an individual package contained an infected bulb (Table 3). The ethylene production of Fusarium oxysporum f. sp. tulipae has been documented (26). Infection led to package ethylene levels of 2-47 µl/liter, while packages without infected bulbs contained less than 2 µl/liter. The floral abortion response of bulbs to ethylene exposure has been shown to vary with cultivar (6). While a 3-5% O_2 exposure caused less floral abortion of 'Kees Nelis' bulbs (as compared to air storage) when exposed to 10 µl/liter of ethylene (22), it cannot be assumed that the package atmosphere could protect the bulbs from the levels reported here. The infected bulbs also could have continued to damage other bulbs once planted, since ethylene up to 10 µl/liter has been measured in the soil atmosphere surrounding bulbs infected with Fusarium oxysporum f. sp. tulipae (25). Ethylene exposure also could have caused the reduced rooting observed (15, 19).

DeMunk has demonstrated susceptibility differences among different tulip cultivars to infection by Fusarium oxysporum f. sp. tulipae (7). These differences appeared to have affected this experiment. However, different inoculation levels in the bulb production fields in the Netherlands may also have been involved. Vanguard treatment of all the bulbs prior to packaging did not prevent the development of Fusarium infection. Damage from the disease has typically been minimized by removal of infected bulbs (5).

It appeared that infection by Fusarium oxysporum f. sp. tulipae was the primary limiting factor in the packaging of certain cultivars. However, the poor flowering of 'Golden Oxford', 'Oxford', and 'Parade' after 4 wks in the package, despite good root growth and the lack of infection, indicated other possible cultivar limiting factors.

Therefore, selection of bulbs free of infection and proper choice of cultivar will both be necessary for successful packaging.

Temperature Fluctuation Study. The permeabilities to CO_2 and O_2 of LDF-301 film at 3 temperatures are shown in Table 4. A temperature increase from 15 to 25° resulted in a 63% increase in CO_2 permeability and an 82% increase in O_2 permeability. Arrhenius plots of the permeability constants further elucidated the temperature response and allowed calculation of activation energies (E_a) of the permeation process (Figure 3). Karel (16) has indicated that discontinuities in the slope of the Arrhenius plots, which would indicate changing E_a over temperature, are very rare for polymeric films. The higher calculated activation energy for O_2 than for CO_2 permeation indicated that O_2 permeation would change more than the CO_2 permeation for any given temperature change.

The respiratory rate change of the bulbs in response to temperature change also affected the package atmosphere. Figure 4 depicts the CO_2 and O_2 levels in packages during 1 wk at 20° followed by 3 wks at a constant 15, 20, or 25°. Package O_2 level was increased by a temperature drop to 15° while an increase to 25° resulted in little O_2 level change compared to a constant 20°. Bulb O_2 consumption likely increased in response to temperature increase. While a Q_{10} of respiration of precooled tulip bulbs has not been published, an assumption near the 2-3 range would be reasonable (9). The lack of significant package O_2 decline at 25° was likely due to the increased film permeation. However, temperature decline to 15° apparently lowered bulb O_2 consumption more than it decreased film

Table 4. Permeabilities to CO_2 and O_2 of LDF-301 low density polyethylene film at 15, 20, and 25°C.[z]

Temp. (°C)	Permeability (liter·atm^{-1}·day^{-1}·m^{-2})[y]	
	CO_2	O_2
15	12.38 (0.37)	2.82 (0.17)
20	16.43 (0.53)	4.17 (0.12)
25	20.22 (0.62)	5.14 (0.21)

[z]Film supplied by Dow Chemical, U.S.A. Film thickness was 0.051 mm.

[y]Values are means (± 1 SD) of three determinations.

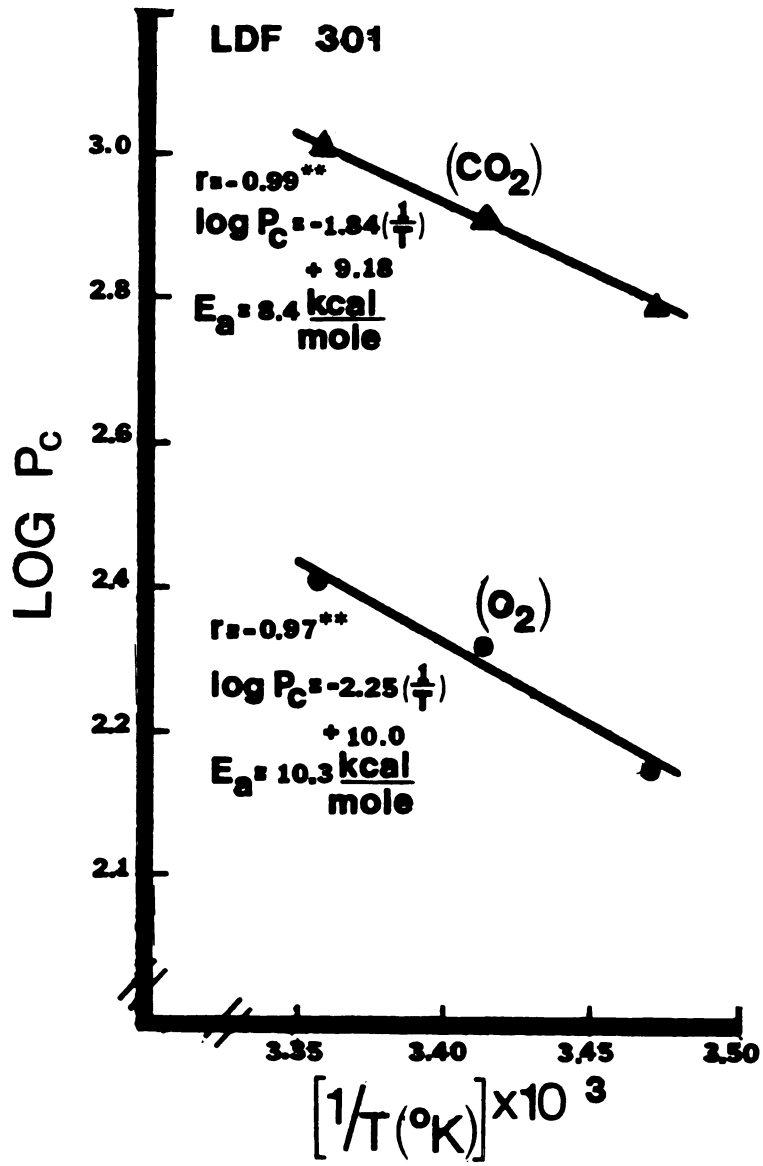


Figure 3. Arrhenius plots of the permeability constants (P_c = ml·mm·atm^{-1}·day^{-1}·m^{-2}) of LDF-301 film to CO_2 and O_2 permeation. (Slope of lines (x 10^3) = $-E_a/2.3R$. Values plotted are means of three determinations. (**) indicates significant r at 1% level.)

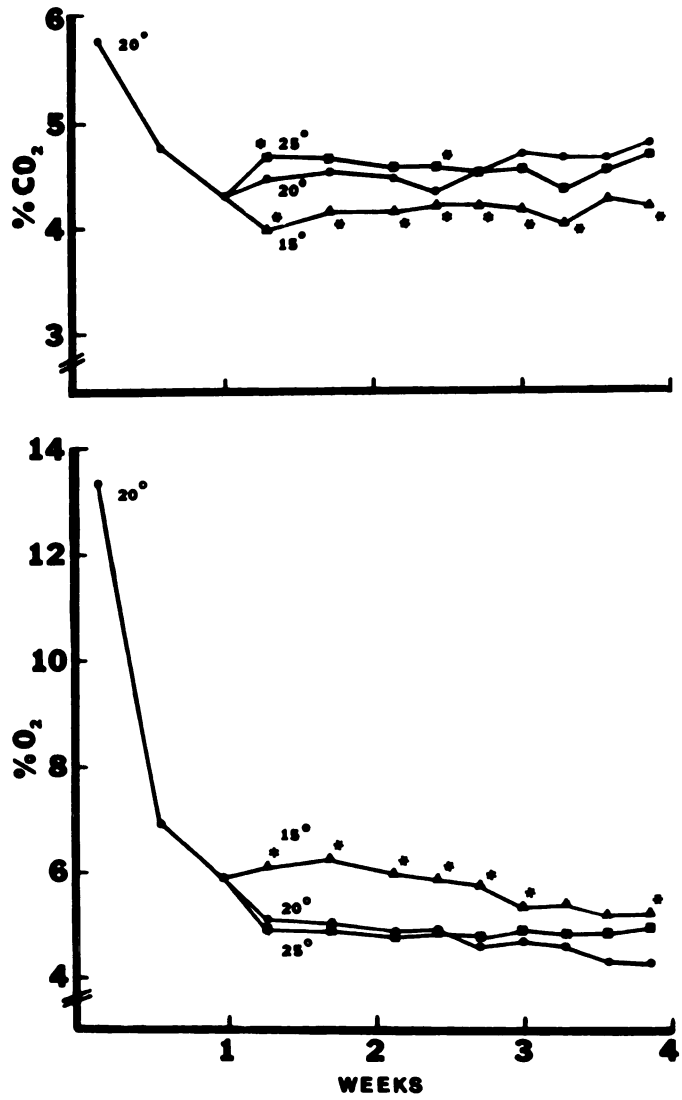


Figure 4. CO_2 levels (top) and O_2 levels (bottom) in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs during storage for 1 wk at 20°C followed by 3 wks at 15, 20, or 25°C. [(*) indicates significant difference from 20° mean within sampling date by Dunnett's procedure, 5% level.]

permeation, leading to increased package O_2. The difference in package response between the two temperature changes of equal increment appeared due to the logarithmic nature of the film permeability temperature response. A temperature change from 20 to 15° decreased permeation by 32% while a change from 20 to 25° increased permeation by only 23% (Table 4). In addition, the tulip bulbs possibly changed Q_{10} of respiration within the 15-25° interval.

The package CO_2 levels responded in a manner opposite to O_2 (Figure 4). A change from 20 to 15° decreased while 20 to 25° increased the package CO_2 levels, although the latter increase was minor. The demonstrated lower level of temperature responsiveness of film CO_2 permeation apparently caused bulb CO_2 production change to be a more important determinant of package CO_2 level change than was film permeation change.

All package CO_2 and O_2 levels displayed greater variability during the final storage wk. During that same period, the 20° control packages tended toward slightly decreased O_2 and increased CO_2 levels.

Package CO_2 and O_2 levels displayed similar responses to 2 day fluctuations to 15 or to 25° during the second storage wk (Figure 5). Both levels returned to the levels of the 20° continuous controls upon return to 20°. During the last 2 storage wks, only package CO_2 appeared to respond to the temperature decline to 15° while O_2 levels remained unchanged. This suggested that after 2 wks of storage, the O_2 consumption of the bulbs became less responsive to temperature fluctuation while CO_2 production continued to respond. A change in the RQ of the bulbs possibly had occurred in response to continued exposure of the bulbs to the MA conditions. This effect was further

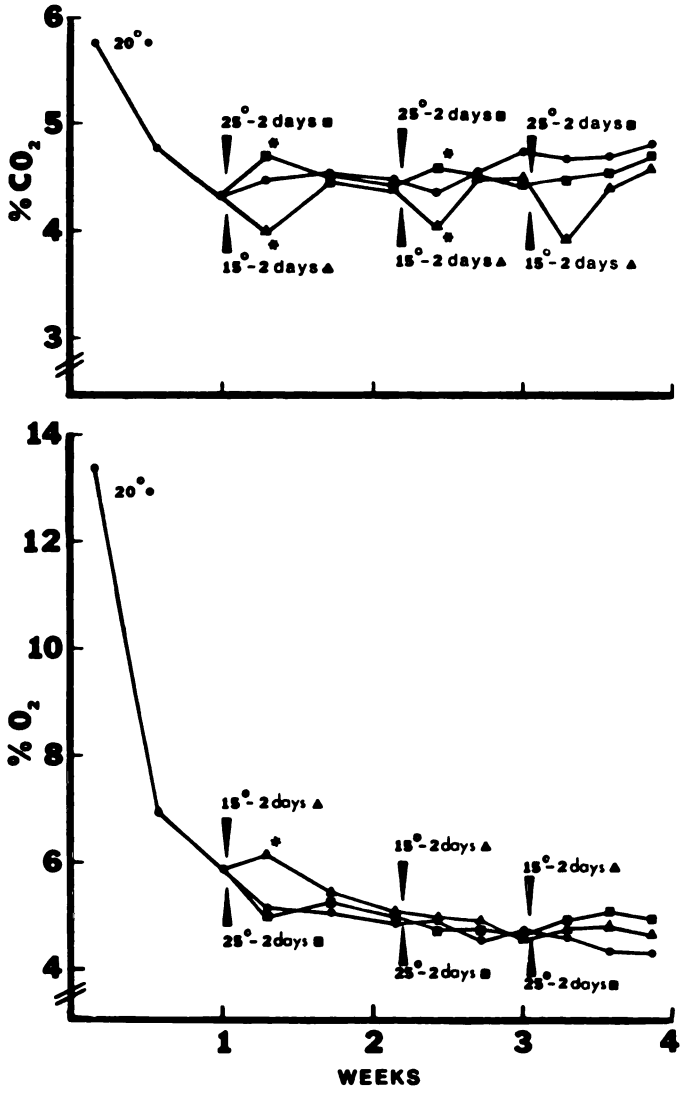


Figure 5. CO_2 levels (top) and O_2 levels (bottom) in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs during storage for 1 wk at 20°C followed by 3 wks at 15° for 2 days/20° for 5 days; 25° for 2 days/20° for 5 days; or continuous 20° storage. [(*) indicates significant difference from continuous 20° mean within sampling date by Dunnett's procedure, 5% level.]

elucidated by the 2 days at 25° followed by 2 days at 15° treatment (Figure 6). No significant effect upon package O_2 level was observed at any time while CO_2 responded as before. Apparently, the 25° exposure before the 15° one yielded enough exposure of the bulbs to the MA conditions to induce the change in RQ and eliminate the O_2 increase at 15° even during the second wk.

An additional effect of 15° exposure was the condensation observed on the inside of the LDF-301 film packages. This disappeared upon return of the packages to 20°. Some root emergence did occur from the bulbs in response to the condensate. However, this emergence did not hinder subsequent rooting or flowering, since damage or desiccation of the roots was avoided during planting.

While package atmospheres did display some statistically significant changes in response to temperature fluctuation, these differences had little practical effect upon subsequent flowering of the bulbs. All packaged and non-packaged bulbs flowered and rooted well after a total of 2 wks at any temperature (Table 5). The LDF-301 film packaging for 3 total wks of storage was successful at all temperatures. At a constant 15° exposure, there was excellent flowering from packaged and non-packaged bulbs alike (Table 6). This indicated that at temperatures near 15°, the package may possibly maintain flowering for periods longer than 4 wks. Rooting was excellent from all packaged bulbs. The average abnormal flower obtained from the non-packaged bulbs had dried and yellow tepals. There were significantly lower root ratings from the non-packaged bulbs than from the packaged bulbs for all but 2 of the temperature regimes.

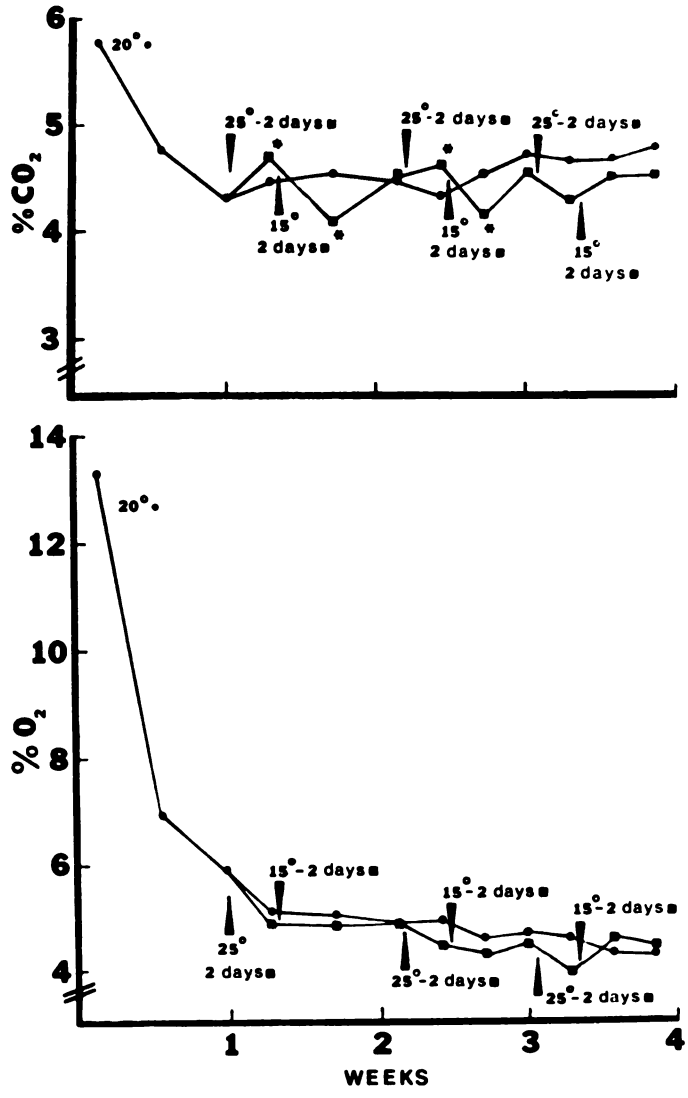


Figure 6. CO_2 levels (top) and O_2 levels (bottom) in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs during storage for 1 wk at 20°C followed by 3 wks at 25° for 2 days/15° for 2 days/20° for 3 days; or continuous 20° storage. [(*) indicates significant difference from continuous 20° mean within sampling date by Dunnett's procedure, 5% level.]

Table 5. Flowering of precooled 'Kees Nelis' tulip bulbs after storage in LDF-301 film packages or non-packaged for 1 wk at 20°C and an additional wk at 6 temperature regimes.[z]

Temperature regime[y]	% normal flowers[x]		Abnormality ratings[w]		Root ratings[v]	
	non-packaged	LDF 301	non-packaged	LDF 301	non-packaged	LDF 301
20° - constant	95	100	---	---	3.8	3.8
25° - constant	85	95	2.0	---	3.6	3.9
15° - constant	95	95	---	---	3.9	3.9
25° - 2 days/wk	95	100	---	---	3.6 *	4.0
15° - 2 days/wk	95	95	---	---	3.7	3.9
25° - 2 days/wk, 15° - 2 days/wk	95	95	---	---	3.8	3.9

[z]Initial post precooled control bulbs yielded 100% normal flowers and a root rating of 3.9.

[y]Bulbs under non-constant temperatures returned to 20°C for the remainder of each wk of storage.

[x]No significant differences between packaged and non-packaged bulbs within temperature regime by Mann-Whitney nonparametric statistic.

[w]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated 0-4 according to symptoms of shoot and floral abnormality (see text).

[v]Root growth from each bulb rated 0-4. (*) indicates difference between packaged and non-packaged bulbs within temperature regime by LSD test at 5% level. (See text for root rating scheme.)

Table 6. Flowering of precooled 'Kees Nelis' tulip bulbs after storage in LDF-301 film packages or non-packaged for 1 wk at 20°C and an additional 2 wks at 6 temperature regimes.[z]

Temperature regime[y]	% normal flowers[x] non-packaged		LDF 301	Abnormality ratings[w] non-packaged	LDF 301	Root ratings[v] non-packaged		LDF 301
20° - constant	5	*	95	1.8	---	3.0	*	3.9
25° - constant	0	*	100	1.7	---	2.4	*	3.9
15° - constant	95		90	---	---	3.6		3.9
25° - 2 days/wk	15	*	90	1.9	2.0	2.9	*	3.8
15° - 2 days/wk	35	*	95	2.0	---	3.2	*	3.9
25° - 2 days/wk, 15° - 2 days/wk	25	*	80	2.4	2.6	3.6		4.0

[z]Initial post precooled control bulbs yielded 100% normal flowers and a root rating of 3.9.

[y]Bulbs under non-constant temperatures returned to 20°C for the remainder of each wk of storage.

[x](*) indicates significant difference between packaged and non-packaged bulbs within temperature regime at 5% level by Mann-Whitney nonparametric statistic.

[w]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated 0-4 according to symptoms of shoot and floral abnormality (see text). There were no significant differences.

[v]Root growth from each bulb rated 0-4. (*) indicates difference between packaged and non-packaged bulbs within temperature regime by LSD test at 5% level. (See text for root rating scheme.)

After 4 total storage wks, packaged bulbs yielded a significantly greater percent of normal flowers than non-packaged bulbs at all temperature regimes (Table 7). However, the constant 25° and 25° for 2 days/wk regimes yielded only 45% and 65% normal flowers respectively. Possibly carbohydrate depletion from higher bulb respiration rates was occurring. All of the packaged bulbs rooted well after 4 wks of storage, while the non-packaged bulbs displayed poorer rooting than after 3 wks of storage (Table 6). Floral abnormalities from the non-packaged bulbs also were more severe after 4 wks. Except for the constant 15° and the 15° for 2 days/wk regimes, packaging for 4 total wks was slightly less successful than for 3 wks and less successful than results obtained with bulbs packaged for 4 wks in another study (Section II). However, these bulbs were packaged on Jan. 27, while bulbs in the previous study were packaged on Dec. 30. The further developed state of the bulbs at the start of precooling and ultimately at packaging could have limited the successful duration of storage. This effect has been observed before with bulbs stored under continuous flow low O_2 atmospheres (22). Other techniques, such as applying low temperatures before the bulb floral organs differentiate (17) may be needed to delay bulb development before precooling. This technique may allow equally successful packaging later in the forcing season.

The results outlined here are further evidence for Henig and Gilberts' suggestion that temperature fluctuation could affect commodity respiratory activity and film permeability to similar degrees, leading to package temperature adaptability (13). In these experiments, temperature change was applied after the packages approached their equilibrium atmospheres. However, application of temperature

Table 7. Flowering of precooled 'Kees Nelis' tulip bulbs after storage in LDF-301 film packages or non-packaged for 1 wk at 20°C and an additional 3 wks at 6 temperature regimes.[z]

Temperature regime[y]	% normal flowers[x]			Abnormality ratings[w,u]			Root ratings[v,u]		
	non-packaged		LDF 301	non-packaged		LDF 301	non-packaged		LDF 301
20° - constant	0	*	80	0.2	*	1.8	1.0	*	3.6
25° - constant	0	*	45	0.6	*	3.2	0.9	*	3.8
15° - constant	5	*	100	0.9		---	1.5	*	3.9
25° - 2 days/wk	0	*	65	0.3	*	3.2	0.5	*	3.9
15° - 2 days/wk	0	*	95	0.1		---	0.4	*	3.9
25° - 2 days/wk, 15° - 2 days/wk	0	*	80	0.3	*	2.8	1.1	*	3.9

[z]Initial post precooled control bulbs yielded 100% normal flowers and a root rating of 3.9.

[y]Bulbs under non-constant temperatures returned to 20°C for the remainder of each wk of storage.

[x](*) indicates significant difference between packaged and non-packaged bulbs within temperature regime at 5% level by Mann-Whitney nonparametric statistic.

[w]Ratings indicated only when at least 2 reps (pots) contained abnormal flowers. Each abnormal plant rated 0-4 according to symptoms of shoot and floral abnormality.

[v]Root growth from each bulb rated 0-4 (see text).

[u](*) indicates difference between packaged and non-packaged bulbs within temperature regime by LSD test at 5% level.

change before this time has been shown to alter only the time to obtain equilibrium in a sealed package (13) due to the altered respiration rate of the commodity. Exposure of the package to 25° appeared to limit the successful storage period and is not recommended. However, extended periods of 25° seem unlikely in the retail marketplace during the winter and early spring. Therefore the MA package of precooled tulip bulbs will maintain bulb flowering potential under reasonable marketplace temperature fluctuations around 20° without anaerobic package atmospheres occurring.

Bulb Organ Study. The changes in fresh weight (FW), dry weight (DW), and fresh/dry weight ratio (FW/DW) of the various bulb organs of packaged and non-packaged bulbs through 4 wks of storage are shown in Figure 7. Significant linear duration x treatment interactions for total bulb FW and DW indicated a linear loss of ca. 30% of the FW and 25% of the DW from non-packaged bulbs during the 4 wks. The DW loss prevention in packaged bulbs was likely due to lowered respiration rate of the bulbs under the MA conditions, while the FW loss prevention was due to the saturated RH package conditions.

The bulb scales behaved similarly, with 35% DW and 45% FW loss from non-packaged bulbs compared to packaged bulbs. Additionally, a significant linear duration x treatment interaction for FW/DW indicated some desiccation of the scales that was prevented by the packaging. The same interaction elucidated a linear 20% loss in shoot DW and a linear 38% loss in shoot FW from non-packaged bulbs that did not occur in packaged bulbs. Packaged bulbs generally had higher shoot FW/DW than non-packaged bulbs although no duration effect was seen.

Figure 7. Dry weights (solid bars), fresh weights (hollow bars), and fresh/dry weight ratios (numbers above bars) of various organs of precooled 'Kees Nelis' tulip bulbs stored in the open (O) or in LDF-301 film packages (P) through 4 wks at 20°C. (Treatment/duration effects upon the fresh weight, dry weight, or fresh/dry weight ratio of each bulb organ significant at 5% (*) and 1% (**) levels indicated by lower case letters to the right of corresponding 4 wk bars or ratios according to the following scheme:

Main Effects

(a) treatment (package vs. open storage)

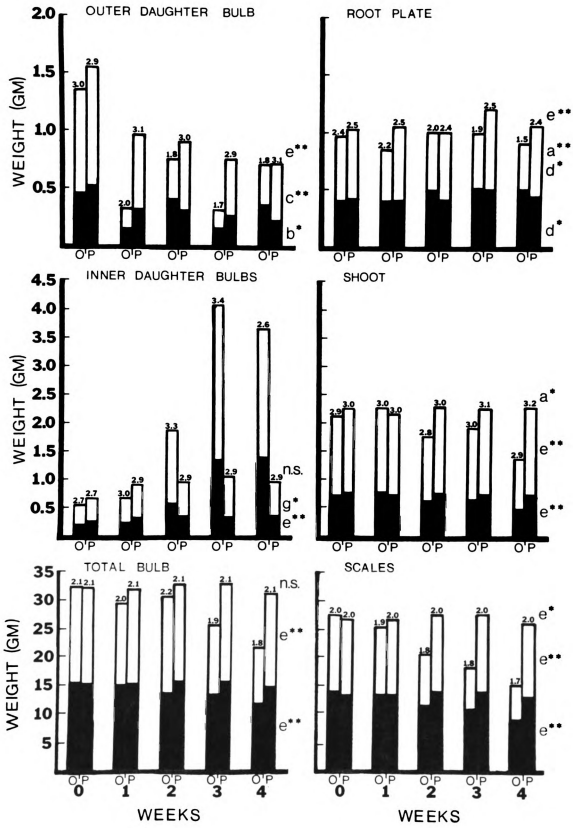
duration (0-4 wks)
(b) linear
(c) quadratic
(d) cubic

Interactions

(e) linear duration x treatment

(f) quadratic duration x treatment

(g) cubic duration x treatment)


OUTER DAUGHTER BULB
ROOT PLATE
INNER DAUGHTER BULBS
SHOOT
TOTAL BULB
SCALES
WEIGHT (GM)
WEEKS
O'P
0
1
2
3
4
n.s.
a*
a**
b*
c**
d*
e*
e**
g*

While the analysis elucidated some main effects for FW and DW of the outer daughter bulb and the root plate, the changing FW/DW appeared most meaningful. Interaction again displayed a linear decline in FW/DW of 37% for these organs from non-packaged bulbs that was prevented by the packaging.

The inner daughter bulbs displayed the most profound changes of all the bulb organs. Packaging maintained fairly consistent FW and DW through 4 wks. Daughter bulb DW increased linearly through 4 wks when non-packaged. A significant cubic duration x treatment interaction for FW indicated a peaking of FW at 3 wks followed by a slight decline for non-packaged bulb inner daughter bulbs. The 7-fold increase in DW and FW of the inner daughter bulbs indicated significant daughter bulb growth in non-packaged bulbs that was prevented by the packaging.

These results suggested that packaging has many effects upon the precooled tulip bulbs. Prevention of desiccation, especially of the root plate and scales, is a significant aspect of this packaging system. However, root plate hydration maintenance was not the primary package function. Previous studies have shown that non-humidified, continuous flow, low O_2 atmospheres increased bulb flowering ability (22). This suggests that the reduced O_2, and possibly the elevated CO_2, in the package are critical to the package function.

The prevention of daughter bulb enlargement was another benefit of the packaging system. However, it was not clear as to whether the daughter bulb enlargement led to floral abortion of non-packaged bulbs or was just a symptom of the loss of floral shoot apical dominance. DeMunk and Gijzenberg (8) have suggested that the floral shoot competes with the daughter bulbs for available substrate. They added that

substrate distribution may be determined by the shoot hormonal status. Cytokinins and giberellins strengthened the sink strength while ethylene exposure weakened it. The low O_2 and elevated CO_2 package atmosphere could have countered ethylene effects upon the shoot (2), allowing it to remain a strong sink, leading to flowering and prevention of daughter bulb enlargement during storage. Accordingly, the production of ethylene by the non-packaged bulbs (20, 23) could have led to decreased shoot sink strength, daughter bulb enlargement, and ulitmately floral abortion.

LeNard (18) has outlined the promotive effects of the cold treatment upon both daughter bulb enlargement and shoot elongation. He suggested that daughter bulb enlargement must not exceed growth of the shoot or abortion will result. Ho and Rees (14) have shown that upon completion of the cold treatment and transferral to 18°, the growing leaves supply carbon for the flower, stem, and daughter bulbs. At this point, the daughter bulbs were the only organs still receiving carbon from the scales. While growth of the shoot did not occur in this experiment during storage, the removal of the bulbs from precooling possibly triggered a reduced shoot sink strength while the daughter bulb sink strength was maintained. The enlargement of the daughter bulbs of non-packaged bulbs could then have led to floral abortion. Complex energy-requiring carbohydrate transformations occur in the bulb scales before translocation to the various organs (1, 10, 21). The package atmosphere may have slowed this process by reducing the available metabolic energy and thereby reducing daughter bulb enlargement.

Rees and Charles-Edwards (24) have speculated that increased soluble sugar content of the floral shoot in response to cooling results in water uptake and high shoot turgor pressure, which lead to shoot elongation. A high respiration rate in non-packaged bulb shoots could have lowered the sugar content leading to lower turgor (decreased FW/DW); while the packaged bulb shoots, with lowered respiration rates, maintained high shoot turgor leading to elongation and flowering upon planting. This experiment did not elucidate which, if any, of the above mechanisms was critical to the success of the MA package.

In conclusion, the storage of many cultivars of precooled tulip bulbs in MA packages was limited by infection of the bulbs by Fusarium oxysporum f. sp. tulipae, due to ethylene production by the fungus. Packages of 'Kees Nelis' bulbs exposed to temperature fluctuation between 15 and 25° exhibited little change in O_2 or CO_2 levels, while maintaining bulb flowering ability. Package temperature adaptability appeared due to temperature dependence of film permeabilities to CO_2 and O_2. The MA package reduced the total fresh and dry weight losses from the bulbs and prevented daughter bulb enlargement.

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SECTION IV

PATHOGENICITY, FUNGICIDE RESISTANCE, AND ETHYLENE PRODUCTION OF *PENICILLIUM* SPP. ISOLATED FROM TULIP BULBS

Abstract. 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 root plates of precooled tulip bulbs. One isolate of P. corymbiferum displayed benomyl resistance when cultured on benomyl amended potato-dextrose agar (PDA). Vanguard and prochloraz controlled growth of this isolate. The benomyl resistant isolate produced 1.5 $\mu l/cm^2 \cdot hr$ of ethylene when grown on PDA. The other isolates appeared not to produce ethylene on this medium. Ethylene levels remained similar for 2 wks when bulb root plates were inoculated with an ethylene producing (benomyl resistant) or a non-ethylene producing (benomyl susceptible) isolate of P. corymbiferum and subsequently sealed in LDF-301 film packages. However, during a third wk the ethylene producing isolate yielded higher package ethylene levels. Inoculation of benomyl pre-treated excised root plates with a mixed spore suspension of the same 2 isolates yielded a dominance of growth of the resistant isolate which was evident upon reisolation after 1 wk. Vanguard pretreatment controlled infection from both isolates. Infection resulting from mixed isolate inoculation of non-fungicide pretreated root plates appeared less severe than infection from separate isolate inoculations, suggesting a negative synergistic interaction.

Penicillium spp. infection of the root plates of precooled tulip bulbs in sealed modified atmosphere (MA) packages has led to rot of the root plate tissue (Section II). Infection was shown to increase ethylene and CO_2 and decrease O_2 levels in the packages. Reduced root growth and increased floral abortion resulted during subsequent forcing of the bulbs. Bulb pretreatment with benomyl or captan did not control infection, while pretreatment with vanguard or prochloraz, two unregistered fungicides, did control infection of the packaged bulbs.

The production of ethylene by many infected plant tissues has been demonstrated (1, 23). However, since ethylene production by many of the infectious organisms themselves also has been observed (10, 17), the ethylene levels reported in packages of infected tulip bulbs could possibly in part be of fungal origin. Ethylene production in vitro by P. digitatum has been extensively investigated (5, 6, 11, 16, 24). However, ethylene production by the two species P. corymbiferum and P. brevicompactum, reported as pathogens on tulip bulbs (3, 9, 12), has not been investigated. Isolates of P. corymbiferum and P. brevicompactum also have demonstrated resistance to benomyl (3, 9, 12). Therefore, the observed poor control of infection afforded by benomyl pretreatment of bulbs before packaging was not surprising.

The studies reported here investigated the identity, pathogenicity, and virulence of various isolates of Pencillium spp. obtained from tulip bulbs utilized in packaging studies. The growth of these isolates in vitro in the presence of the same fungicides utilized previously as bulb pretreatments was observed. The ethylene production of the Penicillium spp. isolates themselves was measured. The gaseous atmospheres obtained within packages of bulbs infected with an ethylene

producing or a non-ethylene producing isolate were investigated to further elucidate the source of ethylene in infected bulb packages. Finally, the interaction of a benomyl resistant and a non-resistant Penicillium sp. isolate during infection of excised root plates was investigated.

Materials and Methods

Tulip bulbs (12-14 cm in circum.) were shipped to East Lansing, Michigan, from the Netherlands in open tray cases during the 1981-82 and 1982-83 bulb forcing seasons. Temperatures during shipment were 13-17° (1981-82) and 17-20° for 10 days followed by 4 days at 7-15° (1982-83). The shipping/arrival dates were Sept. 11/Oct. 6, 1981, and Aug. 16/30, 1982. All bulbs had reached stage G upon arrival. They were stored at 13° (1981-82) or 17-20° (1982-83) prior to precooling.

The Penicillium isolates utilized for preparation of spore suspensions were cultured from bulbs shipped from the Netherlands on Sept. 11, 1981. The isolates were maintained on potato-dextrose agar (PDA) by repeated culturing at 23-26° and periodic storage at 5°. Sample cultures of 7 isolates were shipped to Dr. H. MisLevick, at the Department of Health and Human Services Laboratory in Beltsville, Maryland, for identification. All spore suspensions or control solutions were prepared with sterile water with afew drops of Tween-20 surfactant to aid in spore distribution. 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.

Isolate Pathogenicity (1981-82). 'Kees Nelis' bulbs were stored at 5° and 80-90% RH from Dec. 21, 1981, to June 8, 1982 (24 wks). Bulbs were removed from storage and the tunics were removed to expose the root plates. Only root plates free of infection by Penicillium spp. were selected and cut from the bulbs. They were cut at the point where the root plate protruded from the scale tissue. The root plates were soaked under gentle agitation for 20 min in 10% bleach (ca. 6000 ppm available chlorine) containing a few drops of Tween-20 surfactant, to destroy any naturally occurring inoculum. After soaking, each root plate was placed cut surface down on plain agar in 100 x 15 mm Petri dishes under aseptic conditions. They were then placed at 23-26° for 2 days which yielded root emergence of ca. 5 mm in length. Subsequently, each root plate was inoculated with a 25 µl droplet of spore suspension (10^3-10^4 spores/µl) of individual Penicillium spp. isolates or with sterile water as a control. Ten root plates (reps) were utilized for each isolate or water control. The Petri dishes were randomly placed on a tray at 23-26°. Each of the ten root plates from each treatment were rated for symptom development after 5, 8, and 11 days. The rating scale was: 0 - no symptoms; 1 - brown discoloration; 2 - moderate infection; 3 - severe infection. The rating was performed blindly, with the experimenter unaware of the isolate or control root plate being rated.

Fungicide Resistance (1981-82). Fungicides tested were: benomyl (Benlate 50 WP) [methyl-1-(butylcarbamoyl) benzimidazole-2 yl carbamate]; vanguard (CGA-64251 10 WG) [1-[[2-(2,4-dichlorophenyl)-4-ethyl, 1,3-dioxolan-2-yl]methyl]-1 H-1,2,4-triazole]; prochloraz (BTS-40 542 40 EC)

[1-(N-propyl-N-(2,4,6-(trichlorophenoxy)ethyl)carbamoyl)imidazole]; and captan 50 WP (N-trichloromethyl-mercapto-4-cylcohexene-1,2-dicarboximide]. Petri dishes (100 x 15 mm) containing PDA or PDA with one of each of the above fungicides were prepared. Fungicide concentrations utilized are shown in Table 2. Spore suspensions (10^3-10^4 spores/µl) were prepared from 13 day old cultures of each isolate. Four spots (40 µl/spot) of each suspension were applied to each prepared dish of PDA. Two Petri dishes (reps) containing 4 spots were utilized for each fungicide/isolate combination. The dishes were placed at 23-26° for 4 days. They were subsequently rated for fungal growth according to the following scheme: (++) - uninhibited growth beyond area of suspension application; (+) - growth limited to area of suspension application; 0 - no growth.

Ethylene Production (1981-82). Seven day old cultures (3-5 cm in diam.) of the Penicillium isolates on PDA in 35 x 10 mm Petri dishes were placed in sterile 473 ml canning jars. The top section of each Petri dish was removed and the jars were sealed and placed at 20° for 10 days. Dishes with uninoculated PDA were sealed into jars as controls. 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. All gas sampling and determination was as previously described (Section I).

Bulb Packaging (1982-83). 'Kees Nelis' bulbs were precooled at 5° and 80-90% RH from Oct. 29, 1982, until Jan. 20, 1983 (12 wks). At the end of precooling, the tunics were removed and only bulbs with root plates free of infection by Penicillium spp. were selected.

The selected bulbs were dipped in 10% bleach for 20 min and then rinsed in sterile water. The base of the bulbs was subsequently dipped for 10 min in sterile water, or a spore suspension of the ethylene producing isolate (isolate 1 - benomyl resistant - 1.8×10^3 spores/µl), or a non-ethylene producing isolate (isolate 2 - benomyl susceptible - 1.4×10^3 spores/µl) of P. corymbiferum. The bulbs then were allowed to dry. They were subsequently sealed in LDF-301 film packages (5 bulbs/package) and stored for 3 wks at 20°. Four replicate packages of each treatment were monitored for CO_2, O_2, and ethylene. The packaging, atmosphere monitoring, and infection evaluation were performed as described in Expt. 1 of Section II.

Pathogen Interaction (1982-83). 'Kees Nelis' bulbs were stored at 5° and 80-90% RH from Nov. 17, 1982, until Feb. 9, 1983 (12 wks). Upon removal from storage, root plates free of infection by Penicillium spp. were selected and cut from the bulbs. They were dipped in 10% bleach for 20 min as described above and subsequently were rinsed twice in sterile water. The root plates then were dipped for 20 min in either benomyl (2000 µg a.i./ml), vanguard (240 µg a.i./ml), or sterile water. Both fungicide suspensions were prepared with sterile water. Three root plates then were placed cut surface down under aseptic conditions on 100 x 15 mm Petri dishes containing plain agar. The dishes then were stored at 23-26° for 2 days. Subsequently, 25 µl of one of 3 different spore suspension or sterile water was applied to each root plate. The 25 µl was dispensed as 3 separate drops spaced equidistantly on the root plate surface. One spore suspension was of the benomyl resistant isolate (isolate 1 - ethylene producing -

1.7 x 10^4 spores/µl) of P. corymbiferum. The second was of the non-resistant isolate (isolate 2 - non-ethylene producing - 1.6 x 10^4 spores/µl). The third suspension was prepared by mixing equal volumes of both suspensions. All suspensions were prepared from 4 wk cultures. All dishes then were placed at 23-26° for 1 wk. The experiment was conducted as a completely randomized 3 x 4 factorial design (3 pretreatments x 4 inoculations including water) with 3 Petri dishes (reps) of each treatment/inoculation combination.

After 1 wk, each of the 3 spots of application on each root plate was rated with the 0-3 infection scale as described above. These 3 ratings then were totalled to yield a possible 0-9 rating for each root plate. The ratings of the 3 root plates then were averaged to yield an infection rating for each replicate Petri dish.

After the rating was completed, reisolation was performed to determine the relative extent of infection due to each isolate. Pieces of tissue were cut from the 3 areas of each of the root plates in each Petri dish. The tissue was pooled and placed in 10 ml of sterile water with 1 drop of Tween-20 surfactant and subsequently vortexed at high speed for 10 sec. A 0.1 ml sample of the suspension was utilized to prepare a 10^0, 10^{-1}, 10^{-2}, and 10^{-3} dilution series. A 0.1 ml sample of each dilution then was spread on PDA in 100 x 15 mm Petri dishes which then were placed at 23-26° for 3 days. The dilution plates that yielded the highest number of distinguishable colonies then were selected for counting. The 10^{-1} or 10^{-2} dilution was counted for the benomyl or water control treatments, while the 10^{-1} dilution was counted for the vanguard treatment. The isolates were identified by previously observed characteristics. The benomyl

resistant isolate appeared blue in the center with white edges on top and white on the bottom. The non-resistant isolate appeared white in the center with green edges on top and yellow on the bottom. Some unidentifiable colonies appeared in the more concentrated dilutions from the water pretreated root plates. These were not included in the total count of resistant and non-resistant colonies.

Results and Discussion

Isolate Identification and Pathogenicity. While all isolates obtained from the tulip bulbs appeared to be pure cultures, 2 were found to be mixed Penicillium spp. isolates upon identification at the Health and Human Services Laboratory. Another isolate was identified as Trichoderma lignorum. These 3 isolates were eliminated from further studies. Three other isolates were identified as P. corymbiferum and a fourth as P. rugulosum. All 4 of these isolates appeared to be pathogenic on tulip bulb root plates (Table 1). P. rugulosum has not previously been shown to be pathogenic on tulip bulbs. One P. corymbiferum isolate (isolate 2) was found to be contaminated with P. variabile, a common soil-borne Penicillium. This contamination appeared not to reduce the virulence of isolate 2. Isolate 1 appeared to infect the root plates more slowly than the other 3 isolates. By day 8, however, all 4 isolates were pathogenic with varying degrees of virulence.

A brown discoloration of the root plate was visible as the first sign of infection for all isolates. This possibly resulted from substances released by the fungal spores. It was difficult to obtain root plates free of any naturally occurring inoculum, even

Table 1. Pathogenicity and virulence of 4 Penicillium spp. isolates on excised root plates of precooled 'Kees Nelis' tulip bulbs.[z]

Isolate	Species	Infection rating[y] Day 5	Day 8	Day 11
1	P. corymbiferum	0.8 ns	1.5	1.8
2	P. corymbiferum	2.5	2.9	2.9
3	P. corymbiferum	2.5	2.5	2.6
4	P. rugulosum	2.1	2.4	2.7
	Control[x]	0.3	0.6	0.6

[z]25 µl of spore suspension (10^3-10^4 spores/µl) applied to each root plate.

[y]Infection rated as: 0 - no symptoms; 1 - brown discoloration only; 2 - moderate infection; 3 - severe infection. Values are means of 10 replicates. Means within column different from control by Mann-Whitney nonparametric statistic, 5% level, except those marked nonsignificant (ns).

[x]Sterile water only applied.

after chlorine treatment. This led to slight infection of a few control root plates. Increased duration of the chlorine treatment or increased chlorine concentrations were not utilized due to the possibility of injury to the root plate tissue. The excised root plates utilized for these pathogenicity tests were obtained from bulbs that were stored at 5° for twice the normal 12 wk period. This may have increased root plate susceptibility to infection. The disease severity may vary with length of precooling and with the date of the start of the precooling period. The high relative humidity conditions established in sealed LDF-301 film packages of tulip bulbs already have been shown as ideal for infection (Section II).

Fungicide Resistance. Growth rate studies of the 4 Penicillium spp. isolates on PDA containing 4 different fungicides indicated that isolate 1 was highly benomyl resistant (Table 2). Pathogenic isolates of P. corymbiferum on hosts have previously been reported as benomyl resistant (3, 12). These data suggest that high levels of infection observed on root plates of tulip bulbs pretreated with benomyl before packaging in other studies (Section II) may have been due to selective growth of this resistant isolate in the presence of benomyl.

An antimitotic mode of action for benomyl and its conversion product methyl benzimidazole-2 yl carbamate (MBC) has been demonstrated (7, 18). Differential binding of MBC to fungal tubulin has been presented as a mechanism of benomyl resistance in Aspergillus nidulans (8). Although this aspect of resistance was not explored in these studies, it is possible that the same mechanism of resistance is occurring with P. corymbiferum and other Penicillium spp.

Table 2. Growth ratings of 4 Penicillium spp. isolates on PDA containing 4 fungicides after 4 days at 23-26°C.

Fungicide	Rate (µg a.i./ml agar)	Growth ratings[z] Isolate[y] 1	2	3	4
Benomyl	500	++	0	0	0
	1000	++	0	0	0
	2000	++	0	0	0
Captan	600	+	+	0	+
	1200	+	+	0	+
	2400	+	+	0	+
Prochloraz	150	0	0	0	0
	300	0	0	0	0
	600	0	0	0	0
Vanguard	60	0	0	0	0
	120	0	0	0	0
	240	0	0	0	0
Control[x]	---	++	++	++	++

[z]Growth rated as: (++) - uninhibited growth beyond area of suspension application; (+) - growth limited to area of suspension application; 0 - no growth. Both replicates of each treatment yielded identical results.

[y]Isolates 1-3 were P. corymbiferum, 4 was P. rugulosum.

[x]Isolates grown on PDA without fungicide.

Isolate 1 did not grow on PDA containing vanguard (19) or prochloraz (22) fungicides. Thus, prevention of growth of this isolate apparently was critical to the infection control afforded by these fungicides on packaged bulbs (Section II). Both of these fungicides have been shown to be active against benomyl resistant P. expansum isolates on stored apples (4).

The slight growth of isolate 1, as well as isolates 2 and 4, on PDA containing captan may have been due to poor penetration of the fungicide into the fungal hyphae (15). The demonstrated mode of action of captan is the formation of thiophosgene within the fungal tissue. The thiophosgene then combines with sulfhydryl groups, inactivating various enzymes. It is unlikely that fungi could develop resistance to this nonspecific mode of action (15). 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. Apparently the captan could penetrate the hyphae of isolate 3 to a greater extent.

Identical results were obtained from fungicide resistance trials performed at the start of the 1982-83 bulb forcing season. These results indicated that changes in fungicide resistance of the isolates during repeated culturing did not occur.

Ethylene Production. The benomyl resistant isolate 1 produced ethylene when grown on PDA, whereas the other tested isolates did not (Table 3). Average production of the 7 day old cultures was ca. 1.5 $\mu l/cm^2 \cdot hr$. This study did not eliminate the possibility of ethylene production by the other isolates on different media or during infection of bulb root plates. The age and growth rate of

Table 3. Ethylene levels accumulated in 473 ml jars containing cultures of Penicillium spp. during 6 days at 20°C.[z]

Isolate	Species	C_2H_4 (µl/liter) 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[x]	0.02	0.03	0.03

[z]Cultures were 7 days old and ca. 3-5 cm in diam. when placed in jars.

[y]Values for isolate 1 significantly different within column from other isolates and control by Mann-Whitney nonparametric statistic, 5% level. Values indicated average ethylene production of ca. 1.5 µl/cm^2·hr.

[x]PDA only.

the cultures also may affect ethylene production. The ethylene production of Mucor hiemalis in culture has been shown to increase with decreasing specific growth rates (14). Therefore, the other isolates may possibly produce ethylene at a later growth stage.

The relationship of the fungal ethylene production observed in this study to the ethylene levels in packages of infected bulbs was difficult to quantify. Atmospheric levels in the jars were ca. 3-9% CO_2 and 15-20% O_2 at the conclusion of the study. The atmosphere in sealed packages of bulbs has been shown to equilibrate near 4% CO_2 and 5% O_2 (Section II). The rate of ethylene production by Fusarium oxysporum f. sp. tulipae, another pathogen of tulip bulbs, has been shown to be dependent upon O_2 level (21). Therefore, the package atmosphere possibly slowed ethylene production by P. corymbiferum on the bulb root plates. However, it is likely that isolate 1 did produce some ethylene when infecting packaged bulbs. The dominance of this isolate could have yielded the high package ethylene levels observed after benomyl pretreatment of bulbs. Penicillium produced ethylene could have ultimately increased the root plate infection by lowering natural bulb resistance. Ethylene exposure of tulip bulbs has led to lower levels of tulipalin-A, a naturally occurring fungitoxic substance in bulbs (2). High fungal ethylene production also has been correlated with high pathogenicity of some F. oxysporum isolates (20).

The benomyl resistant isolate of P. corymbiferum could become dominant on tulip bulbs shipped from the Netherlands if benomyl is continuously utilized. This could become problematic due to the varied detrimental effects of bulb ethylene exposure during shipment,

storage, and forcing (13). Other fungicides, possibly vanguard and prochloraz, should be further tested as possible fungicide drenches for use in the Netherlands.

Bulb Packaging. Root plate application of a spore suspension of isolate 1 (ethylene producing) or isolate 2 (non-ethylene producing) before packaging of precooled bulbs led to similar package ethylene levels through 2 wks of storage at 20° (Figure 1). However, during the third wk 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. By the end of 3 wks, 96-100% of the root plate surface of the average inoculated bulb was infected with Penicillium spp. In this experiment the control bulbs displayed only traces of infection. The chlorine treatment appeared to give effective Penicillium control. However, all of these bulbs had their tunics removed and were carefully selected for disease-free root plates. This suggested that chlorine treatment could be successful on extremely clean material. However, the cost of this technique on a commercial level would likely be prohibitive.

Several possible reasons exist for the observed pattern of package ethylene. Both isolates could have produced ethylene when infecting the bulb root plate, even though only isolate 1 produced ethylene on PDA. If this occurred, variation in growth rate of the two isolates could have led to the different package ethylene levels during the third wk. The response of the bulbs to infection could have been involved also. The infection of many plant tissues has been shown to increase host ethylene production (1, 23). Therefore,

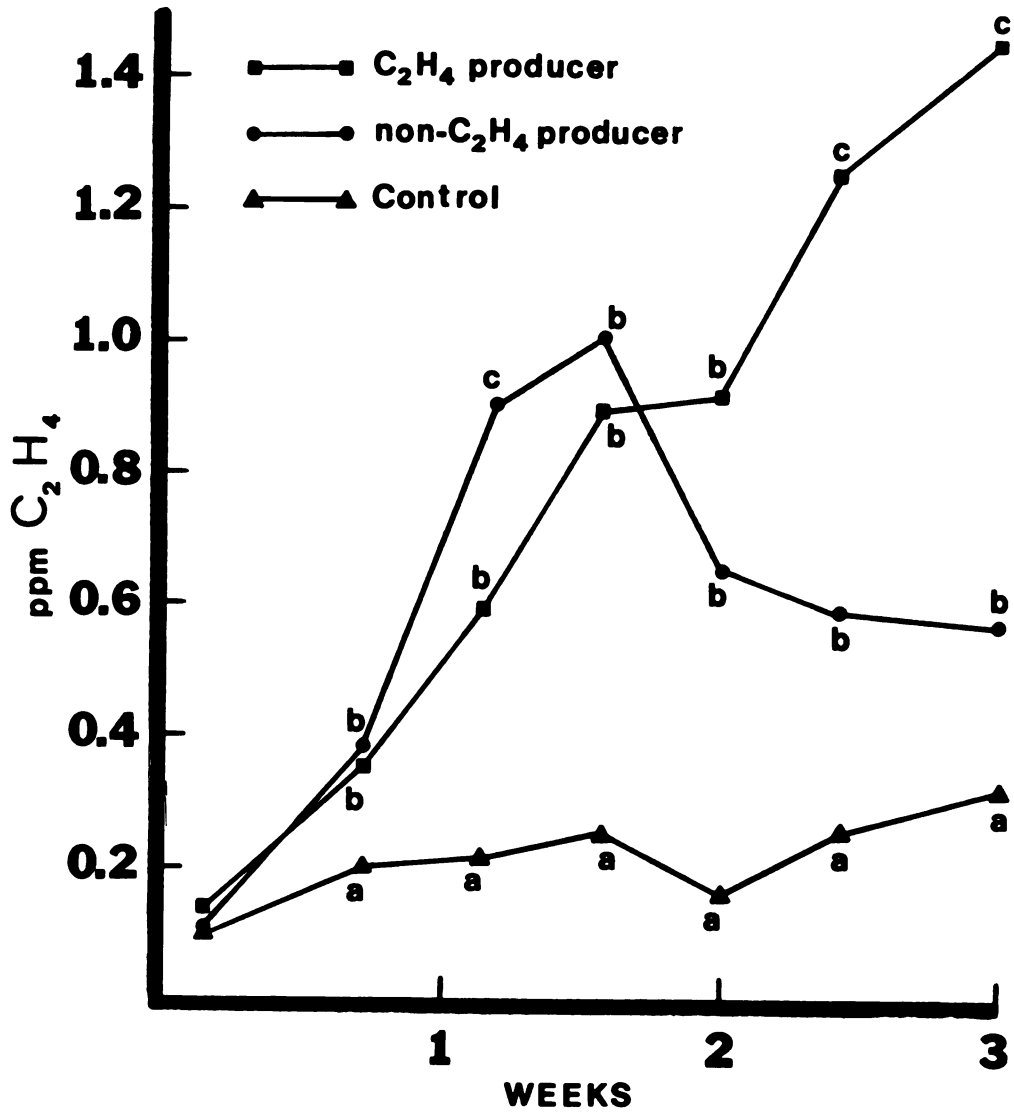

Figure 1. Ethylene levels in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C after tunic removal and root plate inoculation with a spore suspension of an ethylene producing (isolate 1) or a non-ethylene producing (isolate 2) Penicillium corymbiferum. (Control bulb root plates dipped in sterile water. Mean separation within sampling date by Duncan's multiple range test, 5% level.)

the bulbs may have been responsible for at least some of the ethylene production. The fungal ethylene from isolate 1 may have increased during wk 3. Possibly the slowing of the fungal growth rate as the root plate became totally infected by isolate 1 led to the further increase in package ethylene. The source of the ethylene (bulbs or fungi) could not be determined by these experiments.

Inoculation with either isolate yielded similar package O_2 levels (Figure 2). However, the infection resulting from either inoculation led to lower package O_2 compared to control packages. This has been reported and discussed previously (Section II).

Fungal infection of root plates by either isolate also yielded higher package CO_2 levels than control bulbs (Figure 3). However, isolate 2 yielded significantly higher CO_2 levels than isolate 1 during the final storage wk. Since little O_2 change occurred, it appeared that some change in the respiratory quotient of the bulbs and/or the fungus occurred during the final wks.

Pathogen Interaction. Inoculation with the benomyl resistant isolate (isolate 1) led to significantly greater infection than that observed on controls for benomyl pretreated root plates after 1 wk (Table 4). The non-resistant isolate 2 infected benomyl pretreated bulbs at a level intermediate between water controls and the other two treatments. Root plate browning was the major symptom from isolate 2 on the benomyl pretreated bulbs. When both isolates were applied after benomyl pretreatment, significantly greater infection than from control root plates was observed. However, the resistant isolate was the dominant isolate recovered upon reisolation. Dominance

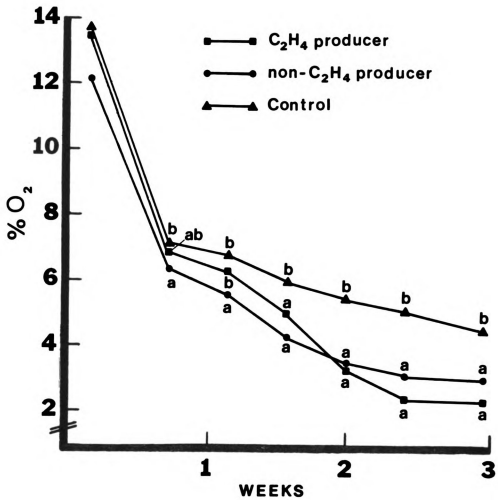

Figure 2. O_2 levels in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C after tunic removal and root plate inoculation with a spore suspension of an ethylene producing (isolate 1) or a non-ethylene producing (isolate 2) Penicillium corymbiferum. (Control bulb root plates dipped in sterile water. Mean separation within sampling date by Duncan's multiple range test, 5% level.)

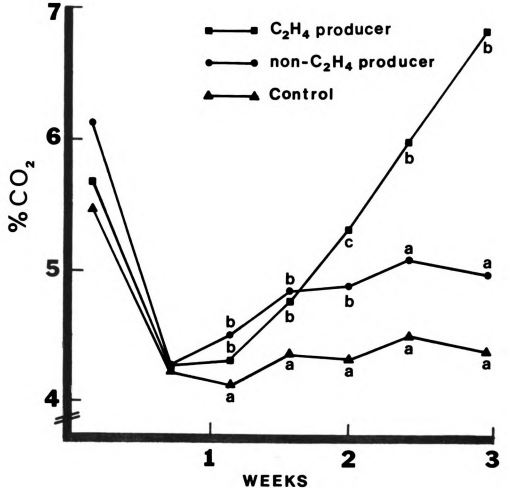

Figure 3. CO_2 levels in LDF-301 film packages of precooled 'Kees Nelis' tulip bulbs through 3 wks at 20°C after tunic removal and root plate inoculation with a spore suspension of an ethylene producing (isolate 1) or a non-ethylene producing (isolate 2) Penicillium corymbiferum. (Control bulb root plates dipped in sterile water. Mean separation within sampling date by Duncan's multiple range test, 5% level.)

Table 4. Infection of excised tulip bulb root plates pretreated with benomyl, vanguard, or water, and subsequently inoculated with a spore suspension of benomyl-resistant (R), non-resistant (NR), or mixed isolates (R+NR) of Penicillium corymbiferum, or of non-inoculated controls (C).

Pretreatment	Inoculation[z]	Infection rating[y]	% R / % NR[x]
Benomyl	C	0.0 a	----
	R	1.7 bc	100/ 0
(2000 µg a.i./ml)	NR	0.9 ab	0/100
	R+NR	1.5 bc	96/ 4 **
Vanguard	C	0.0 a	----
	R	0.1 a	100/ 0
(240 µg a.i./ml)	NR	0.3 ab	0/100
	R+NR	0.3 ab	67/ 33 ns
Water	C	0.4 ab	----
	R	2.7 cd	100/ 0
	NR	3.7 d	0/100
	R+NR	1.3 abc	48/ 52 ns

[z]25 µl of suspensions of the benomyl-resistant (isolate 1 - 1.70 x 10^4 spores/µl), the non-resistant (isolate 2 - 1.63 x 10^4 spores/µl), or an equal volume mixture of both isolates was applied to each root plate. Controls had sterile water applied.

[y]Infection rated on 0-9 scale (see text). Mean separation of ratings from any pretreatment/inoculation combination by Duncan's multiple range test, 5% level.

[x]Percentages calculated on basis of total R and NR colonies reisolated. For mixed culture inoculation, (**) indicates difference between % R and % NR at 1% level by paired t-test. (ns) indicates difference is nonsignificant.

of this isolate probably led to the high infection level observed on packaged benomyl pretreated bulbs (Section II), although more than 2 isolates were likely present on the bulbs.

Little disease was observed on vanguard pretreated root plates. The reisolation percentages likely reflected the spores surviving on the surface of the root plate from the inoculation, since no sporulation was visible. These results demonstrate that vanguard controls root plate infection caused by the benomyl resistant isolate of P. corymbiferum. Few symptoms were observed on water pretreated control root plates. These were likely due to natural inoculum that was not destroyed by the chlorine treatment. Both isolates, when applied separately, were significantly pathogenic on root plates that were water pretreated. There was an intermediate amount of disease on water pretreated root plates inoculated with the isolate mixture. Equal percentages of both isolates were reisolated from the root plates. Possibly a negative synergistic effect between isolate 1 and isolate 2 led to the intermediate infection level. This effect possibly could decrease root plate infection on packaged bulbs, providing a single isolate is not dominant on the bulbs.

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