13. Looney, N.E. and D.L. McIntosh. 1968. Stimulation of pear rooting by pre-plant treatment of nursery stock with indole-3-butyric acid. J. Amer. Soc. Hort. Sci. 92:150-154.

14. Romberg, L.D. and C.L. Smith. 1938. Effects of indole-butyric acid in the rooting of transplanted pecan trees. J. Amer. Soc. Hort. Sci. 36:161-170.

15. Stone, E.C. and G.H. Schubert. 1959. Root regeneration by ponderosa pine lifted at different times of the year. Forest Sci. 5: 322-332.

16. Struve, D.K. 1976. Root regeneration in Scarlet Oak. MS Thesis. Rutgers-The State University of New Jersey, New Brunswick.

17. Watson, G.W. and E.B. Himelick. 1982. Root distribution of nursery trees and its relationship to transplanting success. J. Arboric. 8:225-229.

Factors Affecting Colonization of English Boxwood by *Paecilomyces buxi* Related to Root and Stem Rot Development[1]

LA. Bower,[2] W.H. Wills[3] and R.C. Lambe[3]
Department of Plant Pathology and Physiology
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

Abstract

English boxwood (*Buxus sempervirens* var. *suffruticosa* L.) plants were grown in the greenhouse in association with rhizomes of bromegrass and bindweed. Rhizomes of both species were colonized by *Paecilomyces buxi*. Boxwood plants showed symptoms similar to decline of field grown plants, and boxwood roots were colonized by *P. buxi*. Rooted cuttings of boxwood subjected to temperatures gradually reduced to -5C (23F) were uniformly colonized by *P. buxi* in basal stem lesions where the fungus sporulated at the surface of the stems. Some implications for root-rot development and spread of *P. buxi* related to environmental stress factors are suggested.

Index words: English boxwood decline, *Bromus inermis, Convolvulus sepium,* low temperature stress

Introduction

English boxwood has been grown as an ornamental in the middle Atlantic states since its introduction during Colonial times. Prized for its slow and luxuriant growth in the landscape, English boxwood has relatively few pests or diseases (6). However, Phytophthora root rot and English boxwood decline currently threaten boxwood culture in Virginia.

English boxwood decline was perhaps first described over 50 years ago and has been attributed to many causal factors, including fungi, nematodes, and weather (1, 2, 3, 5). Montgomery *et al.* (4) described the present decline problem and consistently associated the fungus *Paecilomyces buxi* with decline. The total etiology has not been described.

The purposes of these studies were to determine some effects of low temperature stress on colonization of English boxwood by *P. buxi* and on root rot development. In addition, the possible role of two perennial rhizomatous weeds, *Bromus inermis* Leyess (bromegrass) and *Convolvulus sepium* L. (bindweed), in boxwood decline was examined. Both weeds were observed growing in close association with declining English boxwood.

Materials and Methods

Plants used in this research were propagated from cuttings collected from established landscape English boxwood in Colonial Williamsburg, VA, where decline has not been observed. Rooted cuttings were transplanted to a steam pasteurized medium of topsoil and composted ground pine bark (1:1 by vol) in 946 ml (one quart) plastic containers. Osmocote 14N-6.02P-11.6K (14-14-14), 4N-3.87P-2.5K (4-9-3) fertilizer, and ground dolomitic limestone were added to the potting mixture at 85 g (3 oz) ea per .057m^3 (2ft^3). Plants were grown in the greenhouse on wire benches, watered daily, and fertilized monthly with 30 g/l of Peter's 20N-8.6P-16.6K (20-20-20) soluble fertilizer.

Bromegrass or bindweed rhizomes collected from a field in which English boxwood had died were planted with the boxwood as one 2-3 cm long slip per pot of actively growing rhizome in containers. The boxwood were grown in association with the bromegrass or bindweed for 13 mo in the greenhouse. At that point the boxwood was growing in a mat of bromegrass or bindweed. The boxwood plants were then removed from the

[1]Received for publication February 15, 1985. Contribution *487,* Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University.

[2]Former Graduate Research Assistant; President address—Department of Plant Pathology, University of California, Riverside, CA 92521. A portion of the senior author's thesis submitted to the faculty at VPI and SU. Support of the Westmoreland Davis Memorial Foundation, Leesburg, VA is gratefully acknowledged.

[3]Professor and Associate Professor of Plant Pathology, resp.

Table 1. Disease ratings and isolation of *Paecilomyces buxi* from roots and stems of English boxwood, bromegrass, and bindweed grown in close association.

	Test 1			Test 2		
Host	Root rating	Top rating	Percent isolation	Root rating	Top rating	Percent isolation
English boxwood with bromegrass	2.6a[z]	3.0a	44	1.2b	2.0a	85
English boxwood alone	1.0b	1.0b	0	1.0b	1.0c	50
Bromegrass			52			50
English boxwood with bindweed				2.0a	1.7b	80
Bindweed						100

[z]Rated visually on a scale of 1 to 5, healthy to dead. Mean separation within columns followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test. Means are an average of 10 replicates.

containers, the root system washed free of growing medium and visually rated for discoloration and evidence of rot. Tops were also rated for color and size. Root samples from boxwood, bromegrass and bindweed were surface sterilized and plated onto a medium selective for isolation of *Paecilomyces buxi.* The medium consisted of Difco corn meal agar supplemented with soluble starch (10 g/l), yeast extract (100 mg/l), chlorotetracycline (100 mg/l), streptomycin sulfate (100 mg/l) and pimaricin (50 mg/l). This medium was developed by the first junior author for this purpose.

Low temperature stress experiments were conducted with rooted cuttings in 473 ml containers in a medium of Canadian sphagnum moss, composted ground pine bark, and Weblite® (1:1:1 by vol). In the first test, 10 plants were placed in a freezer at ambient room temperature and the temperature reduced approximately 5C (9F) per hour from 25C to -5C (77 to 23F). The plants were left overnight in the freezer at ambient air temperature of -5C (23F) and approximate root temperature of 0C (32F). After equilibration to 20C (68F), plants were each inoculated with 25 ml of a spore suspension (1.5 x 10^6 spores/ml) of *P. buxi* drenched into the container and placed into metal trays in growth chambers. Non-inoculated plants were maintained in a separate growth chamber. In a second experiment, plants were stressed as described above and inoculated prior to stressing (treatment one), after stressing (treatment two), stressed but not inoculated (treatment three), and (treatment four) inoculated but not stressed.

Results and Discussion

In the two experiments where English boxwood were grown in association with bromegrass and bindweed the root systems of boxwood were often discolored and some root rot was observed. In general, these root systems were smaller and were discolored compared with boxwood grown alone (Table 1). The tops of boxwood grown with bromegrass or bindweed were markedly reduced in size and did not produce new growth.

Paecilomyces buxi was isolated from roots of boxwood, bromegrass and bindweed (Table 1). Although boxwood were not artificially inoculated, *P. buxi* may have been introduced with the bromegrass or bindweed rhizomes or perhaps by greenhouse contamination. Non-inoculated boxwood plants grown without bromegrass or bindweed in Test 2 also supported growth of *P. buxi.*

Low-temperature-stressed boxwood showed typical root rot and failure of new root development. Stressed plants had sporulating *P. buxi* colonies at the basal end of the stem. Among plants which were inoculated but not stressed only 10% showed *P. buxi* signs (Table 2).

Decline of English boxwood in the landscape may be related to several factors (4). Results of these studies suggest that both biotic and abiotic factors are involved. Stress may influence infection and colonization by *P. buxi.* Test 1, Table 1, data show that bromegrass may have been naturally colonized by *P. buxi,* and that *P. buxi* may have colonized boxwood roots from its base in the bromegrass rhizomes. In Test 2, Table 1, the evidence suggests a similar relationship with bindweed. Disease severity in boxwood in both tests was greater in the presence of the rhizomes of both bindweed and bromegrass than in their absence. This suggested an associative role for these plants in addition to providing an inoculum source, because no artificial inoculation or infestation with *P. buxi* occurred in either test. Other

Table 2. Basal stem infection of English boxwood by *Paecilomyces buxi* following low temperature stress.

Treatment	Percent basal stems exhibiting signs of *P. buxi*
low temperature stress non-inoculated	80a[z]
low temperature stress pre-stress inoculated	100a
low temperature stress post-stress inoculated	100a
inoculated only	10b
non-inoculated, not stressed	0b

[z]Mean separation by the same letter are not signficantly different at the 5% level using Duncan's Multiple Range Test. There were 10 plants in each treatment.

tests showed that bromegrass and bindweed can be colonized by *P. buxi* in vitro and we have observed that naturally occurring bromegrass or bindweed in a northern Virginia nursery were colonized by *P. buxi* (unpublished data). The role of bromegrass or bindweed in decline of boxwood is not clear but may be as a reservoir of inoculum. The long test period (13 mos) would have allowed ample time for natural, and thus uncontrolled, greenhouse contamination of the tests, perhaps explaining why *P. buxi* was isolated from boxwood growing alone in Test 2, even though not from Test 1.

There was an unexpected, but not surprising, result of the low-temperature-induced stress caused by freezing rooted cuttings of boxwood. A highly significant correlation was found between low temperature treatment, either before or after inoculation or even without inoculation, and development of basal stem lesions which contained sporulating colonies of *P. buxi.* Predisposition by this treatment caused proliferation of the pathogen at the surface of the stems in inoculum from either artificial or natural sources. This could occur in normal winter weather in the field. Development of lesions on the stem, typical of those observed in this experiment, is common in the field and could provide the initial steps in disease development and eventually cause root rot. Predisposed stems could also be a source of new inoculum for spread of the fungus to new infection sites among nearby plants. This might provide the mechanism by which winter injury, often suspected but never proved, might influence both subsequent spread of the pathogen and development of a root rot disease.

These studies have shown that rhizomatous weeds may serve as an inoculum reservoir in boxwood when they are colonized by *Paecilomyces buxi.* A mechanism has been postulated by which low temperature induced stress may pre-dispose English boxwood to stem colonization by *P. buxi* followed by proliferation and spread of the fungus, eventually causing root rot and death of individual plants.

Significance to the Nursery Industry

The production of English boxwood by nurserymen in Virginia is currently difficult because little information is available to explain the large losses caused by decline. Some English boxwood nurseries in certain Northern and Central Virginia counties were lost during the past 12 years due to decline caused by *P. buxi* root and stem rot. Previous research has suggested that environmental stress may be an important factor in decline. This research provides additional information on the possible role of low winter temperature on the development of decline and spread of *P. buxi.* Although it will be impossible to manipulate optimum temperature for boxwood production under field culture, it may be possible to provide stress-free conditions for successful production in container culture. New technology in the area of cold protection such as microfoam blankets and temporary buildings should relieve stress.

Weed control measures to eliminate perennial weeds as inoculum reservoirs may improve the survival rate of English boxwood grown under field conditions.

Literature Cited

1. Andrus, C.F. 1933. Fungus flora accompanying decline of boxwood. Plant Dis. Rptr. 17:169-170.

2. Lambe, R.C. and Wills, W.H. 1975. Decline of English boxwood in Virginia. Plant Dis. Rptr. 59:105-108.

3. McBryde, M.C. 1933. Preliminary studies in boxwood blight. M.S. Thesis. Virginia Polytechnic Institute, Blacksburg, 26 p.

4. Montgomery, G.B., Wills, W.H. and Lambe, R.C. 1977. Etiology of decline of English boxwood. Plant Dis. Rptr. 61:404-408.

5. Tarjan, A.C. 1950. Investigation of meadow nematodes attacking boxwood and the therapeutic value of sodium selenate as a control. Phytopathology 40:1111-1124.

6. Weiss, F. and St. George, R.A. 1959. Culture, diseases and pests of the boxtree. USDA Farmer's Bull. 1855. 18 p.