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Biological control of annual bluegrass (Poa annua) in putting greens /

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BIOLOGICAL CONTROL OF ANNUAL BLUEGRASS (*POA ANNUA*) IN PUTTING GREENS

A Thesis Presented

by

G. MICHAEL ELSTON

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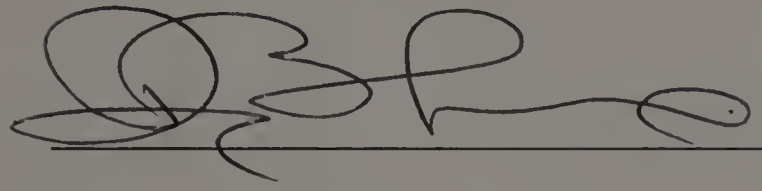

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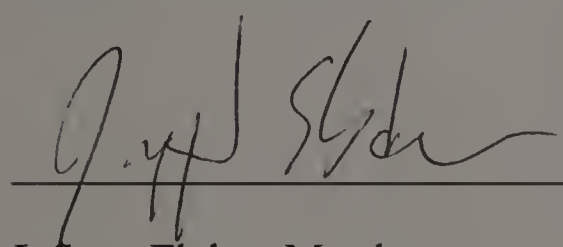

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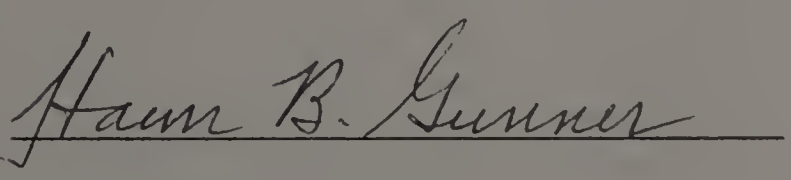

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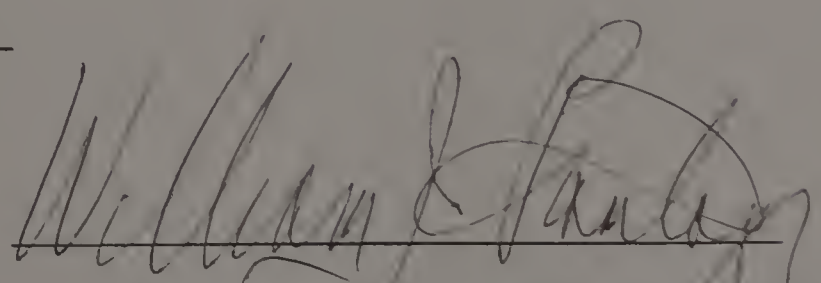

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DEDICATION

I dedicate the completion of this project to my wife, Michelle Poe Elston. Without her, it simply would not be done. She is my teammate and my inspiration. I love you, Michelle, you are my world.

ACKNOWLEDGEMENTS

Without the support and guidance of capable mentors, no one's first piece of research would be worth reading. First and foremost, I would like to thank my advisor, Prof. Prasanta C. Bhowmik. There has been no instance where Dr. Bhowmik did not have time for my questions. He gave me guidance, while at the same time pushing me to think independently, and scientifically. I will always value what I have learned from him, and the friendship we have shared. Many thanks, Doc.

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ABSTRACT

BIOLOGICAL CONTROL OF ANNUAL BLUEGRASS (*POA ANNUA*) IN PUTTING GREENS

MAY 2001

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Annual bluegrass (*Poa annua* L.) is one of the few weeds able to compete in putting greens, where its presence reduces the aesthetic quality and can influence ball roll on the putting surface. Selective herbicides are not available for the control of annual bluegrass in putting greens, so golf course superintendents must use an integrated approach for annual bluegrass management.

Xanthomonas campestris pv. *poannua* has potential as a biological control agent for the selective, postemergence control of annual bluegrass on putting greens. The bacterium enters the plant through the mowing wound, and multiplies rapidly once inside, causing xylem occlusion. The progression of the vascular wilt disease eventually leads to plant death. Desirable turfgrass species are able to fill voids that are created by the declining annual bluegrass. Laboratory experiments were conducted to determine the toxicity of various surfactants, growth regulators, and pesticides, to *Xanthomonas campestris*. Growth room and field experiments were conducted to determine the effects

of various surfactants and plant growth regulators on the activity of *Xanthomonas campestris* in the suppression of annual and perennial biotypes of annual bluegrass. The effects of surfactants, plant growth regulators, and *Xanthomonas campestris* on creeping bentgrass (*Agrostis palustris* Huds.) growth were also examined.

Agri-dex was the only surfactant that was safe to the bacterium at recommended rates. Bifenthrin, was toxic to *Xanthomonas campestris* at recommended rates. *Xanthomonas campestris*, at 10^7 cfu ml^{-1} suppressed both annual and perennial biotypes of annual bluegrass by 100% in the growth room, yet did not suppress annual bluegrass in field trials when applied alone or in combination with surfactants or plant growth regulators. Agri-dex at 0.5% (V/V) accelerated infection of the annual biotype of annual bluegrass by 1 WAT, but did not affect the perennial biotype. Silwet L-77, X-77, and high rates of Break-thru [0.6 and 0.9% (V/V)] reduced the activity of *Xanthomonas campestris* by 23, 35, and 87%, respectively. Break-thru applied at 0.9% (V/V) injured creeping bentgrass.

Paclobutrazol and ECSS 1001 did not affect suppression of the annual biotype of annual bluegrass by *Xanthomonas campestris*, but accelerated suppression of the perennial biotype by 1 WAT. Combining trinexapac-ethyl with the bacterium reduced the activity of *Xanthomonas campestris* in the perennial biotype of annual bluegrass by 50% 7 WAT. The plant growth regulators increased seedhead production by over 30% 4 WAT. Interactions among *Xanthomonas campestris*, annual bluegrass, and the putting green environment must be further examined with regard to survival and growth of *Xanthomonas campestris*, as well as suppression of annual bluegrass.

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MANAGEMENT STRATEGIES FOR ANNUAL BLUEGRASS SUPPRESSION

Introduction

Annual bluegrass, *Poa annua* L. is a highly competitive grass species common to the intensely managed turfgrass of most golf courses in the United States (Branham, 1991). Effective chemical control of annual bluegrass has not been developed, despite the efforts of the herbicide industry. Roberts et al. (1984) isolated and identified the bacterium *Xanthomonas campestris* pv. *poannua* from a diseased annual bluegrass tissue sample collected on a Michigan golf course. Since then, the bacterium has been developed and used as a biological control agent for the selective postemergence control of annual bluegrass in turfgrass.

A great deal of research has been conducted to develop practical means of employing the pathogen. Much has been learned about host-pathogen interactions, but substantial obstacles continue to limit the practicality of using *Xanthomonas campestris* pv. *poannua* as a biological control agent against annual bluegrass infestations on creeping bentgrass (*Agrostis palustris* Huds.) golf greens. One such obstacle has been a differential response of annual (*P. annua* var. *annua* L. Timm) and perennial [*P. annua* var. *reptans* (Hauskins) Timm] biotypes of annual bluegrass. Control levels of the perennial biotype of annual bluegrass, the predominant biotype in New England, have been consistently lower than its annual counterpart.

Biology of annual bluegrass (*Poa annua* L.)

Description

Poa annua L., known as annual bluegrass, annual meadowgrass, or wintergrass, is a member of Poaceae, the grass family. Annual bluegrass occurs in nature as a winter annual (*Poa annua* var. *annua* L. Timm) or perennial [*Poa annua* var. *reptans* (Hauskins) Timm]. The fibrous root system is typically shallow - less than 5 cm deep - but can develop to 25 cm deep (Sprague and Burton, 1937). It reproduces by seed or seed and stolons. Stems are 5 to 25 cm long, having 2 to 4 nodes. It grows erect to prostrate and sometimes roots at the nodes (mostly perennial biotypes). The leaves of annual bluegrass are typically 3 to 10 cm long, 2 to 4 mm wide. They also tend to be v-shaped, glabrous on both sides, light green to dark green, and wrinkled with a prominent boat-shaped tip. The ligules are 2 to 5 mm long and membranous. The panicle may be 2.5 to 10 cm in height with three to six flowers per spikelet. The fruit is an elliptical caryopsis, amber in color, 1.5 to 2.5 mm long, and there are roughly 80 fruits per inflorescence. The species *Poa annua* includes diploid and tetraploid individuals (Ruemelle et al., 1997; Mitich, 1998).

The species is most distinguished from other grasses by its leaves for their combination of wrinkled margins and boat-shaped tips. However, substantial morphological variability exists among individuals of the species (Mitich, 1998; Eggens and Charbonneau, 1992; and Reicher et al., 1998).

History

Poa annua is believed to have originated in the Mediterranean region from a cross between *P. supina, P. infirma,* and one other *Poa* species (Mitich, 1998). At least one of these species is believed to be annual and at least one is believed to be perennial (Eggens and Charbonneau, 1992), which may account for the variation in life cycles among annual bluegrass individuals. From Europe, it is believed to have spread currently to 80 countries, probably through human activities (Mitich, 1998).

Habitat

Climatic requirements. Annual bluegrass is adapted to temperate and alpine biomes, however, it is most productive when temperatures are between 15 and 17 C (Goussoin, 1988). This explains the competitive nature of the weed during the cool months of spring and fall. It does not tolerate extremes of either cold or warm temperatures and is therefore confined, in tropical regions, to mountainous areas (Mitich, 1998). Annual bluegrass does not tolerate periods of drought, and when such conditions endure, they can lead to plant death.

Edaphic requirements. Annual bluegrass is adapted to a variety of soil textures and structures, provided adequate moisture is present. It is highly adapted to compacted soil, as evident by its ability to colonize in heavily trafficked areas of golf course turf and even sidewalk cracks. Annual bluegrass prefers neutral to alkaline soils with high levels of nitrogen and phosphorus (Reicher et al., 1998; Mitich, 1998).

Phenology

Annual bluegrass seeds typically germinate in the fall when temperatures fall below 21 C (Reicher et al., 1998). It overwinters in a dormant state and is one of the first grasses to resume growth in the spring. During fall and spring, the relative growth rate of annual bluegrass surpasses that of most other weeds (Radosevich et al., 1997).

Annual bluegrass is day neutral, though both the annual biotypes and the perennial biotypes produce the majority of their flowers during the months of May and June (Mitich, 1998). Viable annual bluegrass seeds can be produced as rapidly as one day after pollination (Branham, 1991). Annual biotypes die soon after seed production, whereas perennial biotypes are capable of surviving the stress of summer as well as producing seeds in small quantities at any point during the growing season (Eggens and Charbonneau, 1992).

Reproduction

Annual bluegrass produces seeds even under conditions that would prohibit other grasses from reproducing. Seed production varies from 1,050 to 2,250 per plant for annual biotypes and up to 13,000 per plant for perennial biotypes. As many as 360 seeds per plant are produced even by those maintained at 0.5 cm mowing height (Mitich, 1998).

Poa annua possesses a great deal of genotypic and phenotypic variation among its progeny through self-fertilization and outbreeding (Ruemelle et al., 1997). While all members of the species share the same general morphological characteristics, numerous biotypes of annual bluegrass exhibit variations in growth habit, leaf color, life cycle, and ploidy level.

The seeds of annual bluegrass are dispersed by a variety of mechanisms. Annual bluegrass seeds are 1.5 to 2.5 cm long and weigh roughly 0.5 mg each, thus they are easily carried by water, shoes, and machinery. Seeds can also be dispersed by birds and livestock (Mitich, 1998).

Population dynamics

Because of its genetic diversity, annual bluegrass has the ability to respond to various selection pressures. Individuals that thrive under a given set of environmental parameters are selected, thus every succeeding generation is better adapted to the same ecological niche that the population occupies. Genotypic differences among populations inhabiting different microclimates (greens, fairways, and roughs) even on the same golf course have been observed. Phenotypic differences were observed between individuals collected from cropland populations and those from golf courses (Lush, 1989).

This plastic nature of the species also influences the manner in which annual bluegrass competes. In cultivated areas, annual biotypes dominate (Mitich, 1998). These populations are r-selected and therefore produce a large number of seeds (Danneberger, 1993).. The annual growth cycle is completed between annual cultivations. Conversely, populations of the perennial biotypes, more commonly associated with pastures and turfgrass environments (Mitich, 1998), are considered K-selected due to a greater resource allocation toward rapid vegetative growth during cooler periods and conditions of compacted soil (Danneberger, 1993).

Economic importance

Annual bluegrass is a highly competitive weed found in temperate and alpine regions of every continent (Mitich, 1998; Eggens and Charbonneau, 1992). It invades cropping systems ranging from pastures and agronomic crops to horticultural crops and intensely managed turfgrass areas (Mitich, 1998). Annual bluegrass impedes the production of food crops such as sugar beets, potatoes, and alfalfa (Mitich, 1998). It is found on virtually every golf course in the U.S. and is a challenging weed to turfgrass managers world wide (Branham, 1991).

Annual bluegrass is the most common weed species to the closely mown turfgrass of golf course greens, tees and fairways. Infestations of the weed reduce both the aesthetic quality and playability of the turfgrasses. The appearance of annual bluegrass is considered undesirable by the golfing community due to the light apple green color of its foliage and the seed heads produced during the spring and early summer. In addition, the plants often die in summer, due to low tolerances to heat, drought, and disease, leaving bare spots in the turfgrass areas. These bare spots are both unsightly and unacceptable to playability of the turfgrass.

In contrast, annual bluegrass is commonly managed as a turfgrass, often due to its invasion of the original species. The natural (wild type) annual bluegrass is considered a low-quality turfgrass due to its many undesirable qualities. The introduction of cultivated types of *Poa annua reptans,* genetically engineered toward darker color, stoloniferous habit, and reduced seed production may have a potential market in the turfgrass industry.

Suppression of annual bluegrass in creeping bentgrass putting greens

Attempts to discover selective annual bluegrass control continue but with little success. Even with a selective herbicide, the bare spots created in many cases would be unacceptable in terms of both aesthetic quality and playability of the putting surface. Therefore the objective of many annual bluegrass management programs is suppression of growth and development, including seedhead production. Suppressing the growth of annual bluegrass provides the bentgrass with a competitive advantage, enabling it to take the weed's place before reductions in overall turfgrass quality occur. Seedhead suppression limits the spread of annual bluegrass, improves the putting quality and turfgrass appearance, and may reduce the magnitude of future infestations of the weed species.

For the suppression of annual bluegrass on bentgrass putting greens, many turfgrass managers rely on an integrated approach that includes cultural, chemical, and recently, biological control strategies.

Cultural

Annual bluegrass not only tolerates, but thrives and reproduces under the stressful conditions of close mowing and soil compaction typical of putting greens. Environmental conditions that favor creeping bentgrass differ only slightly from those which favor annual bluegrass, making it difficult to eliminate the weed without harming the desirable turfgrass. The main differences are that annual bluegrass exhibits a high tolerance of soil compaction, but is sensitive to heat and drought stress and nutrient deficiencies (Reicher et al., 1998; Mitich, 1998).

Compacted soils restrict the development of a deep root system of annual bluegrass as well as desirable turfgrasses. Compaction tolerance of annual bluegrass is attributed to its ability to develop an effective shallow root system (Sprague and Burton, 1937). To limit this advantage, greens are aerified regularly to promote bentgrass root growth. The process is typically carried out in late spring, when bentgrass is actively growing, and annual bluegrass seeds are less likely to germinate, as compared to germination in the fall (Beard, 1973).

The low tolerance of annual bluegrass to drought stress is a result of a shallow root system that enables the species to withstand soil compaction. However, high soil moisture resulting from light and frequent irrigation or rainfall supports the growth of annual bluegrass in putting greens. Conversely, fluctuation in soil moisture that results from deep and infrequent irrigation promotes deeper bentgrass root development. Having a deep root system makes water in lower soil horizons available to only the bentgrass, thus giving bentgrass an advantage over annual bluegrass during periods of drought. Once the bentgrass root system is sufficiently deep, the putting greens are irrigated according to the minimum requirements of the bentgrass - and below the minimum requirements of the annual bluegrass.

The optimum soil fertility level for annual bluegrass is higher than the nutritional requirements of creeping bentgrass. Creeping bentgrass can draw nutrients from a greater volume of soil than annual bluegrass. In addition, pH favoring creeping bentgrass varies from 5.5 to 6.5 (Beard, 1982), whereas annual bluegrass thrives in neutral to alkaline soils (Reicher et al., 1998; Mitich, 1998). Therefore, pH and soil fertility, like

Chemical

Chemical control of annual bluegrass has been inconsistent and unacceptable (Watschke et al., 1979; Gaul and Christians, 1988), and often accompanies bentgrass injury (Johnson and Murphy, 1996; Fagerness and Penner, 1998; Watschke and Borger, 1998). However, several herbicides and plant growth regulators have been marginally effective and are labeled for annual bluegrass control on creeping bentgrass putting greens.

Preemergent herbicides such as bensulide [*O, O*-bis[1-methylethyl] *S*-[2-[(phenylsulfonyl) amino] ethyl] phosphorodithioate] and dithiopyr [*S, S*-dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3, 5-pyridinedicarbothioate are recommended mainly for control of the annual biotypes of annual bluegrass in established stands of creeping bentgrass (McCarty and Murphy, 1994; Bhowmik et al., 1997). Taylorson and Spak (1994) reported that ethofumesate [(±)-2-ethoxy-2, 3-dihydro-3, 3-dimethyl-5-benzofuranyl methanesulfonate], applied at 0.85 kg ha^{-1} preemergence or early postemergence, controlled both biotypes of annual bluegrass up to 94% with no injury to the bentgrass. Gaul and Christians (1988) found that chlorsulfuron [2-chloro-*N*[[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)amino]carbonyl] benzenesulfonamide], when applied postemergence to annual bluegrass, proved effective in controlling two of the three annual bluegrass biotypes tested, and had no effect on creeping bentgrass.

Annual bluegrass growth and seedhead suppression has been accomplished with various growth regulators such as paclobutrazol [(±)-(R*,R*)-§-[(4-chlorophenol) methyl]-a-(1,1 dimethyl)-1H-1,2,4-triazole-1-ethanol], melfluidide [*N*-[2, 4-dimethyl-5-

Annual bluegrass growth and seedhead suppression has been accomplished with various growth regulators such as paclobutrazol [(±)-(R*,R*)-§-[(4-chlorophenol) methyl]-a-(1,1 dimethyl)-1H-1,2,4-triazole-1-ethanol], melfluidide [*N*-[2, 4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide] (Watschke and Borger, 1998), with inconsistent results (Watschke et al., 1979; Watschke and Borger, 1998) and with bentgrass injury (Johnson and Murphy, 1996).

Biological control of annual bluegrass (*Poa annua* L.)

Xanthomonas campestris is a widely studied species of bacteria primarily because of its many phytopathogenic varieties, or pathovars. Roberts et al. (1981) isolated a strain of *Xanthomonas campestris* that was found to incite a bacterial wilt disease in annual bluegrass, and subsequently named the pathovar *"poannua"*. Since then, isolates of *Xanthomonas campestris* pv. *poannua* have been selected and developed for use as bioherbicides for annual bluegrass control in turfgrass. The Michigan strain, MB 218, has been registered as such and is currently being developed under the trade name XPo®. Isolates of a similar, if not identical (Nishino et al., 1995), *Xanthomonas campestris* pathovar were isolated and selected for development in Japan (Imaizumi et al., 1997). As a result, *Xanthomonas campestris* pv. *poae*, isolate JT-P482, (also referred to as *Xanthomonas translucens* pv. *poae*) is currently registered in Japan (CAMPERICO®).

Microbial properties of *Xanthomonas campestris*

Swings et al. (1993) described colony morphology of *Xanthomonas campestris* as circular with smooth margins, convex, pale yellow, and mucoid. The species is gram-

Host specificity

Xanthomonas campestris pv. *poannua* is selective toward annual bluegrass. The symptoms associated with *Xanthomonas campestris* have not been observed in other plants inoculated with the bacterium (Savage, 1990). Imaizumi et al. (1997) reported U Uthat *Xanthomonas campestris* did not produce any symptoms in creeping bentgrass (*Agrostis palustris* Huds.) or Kentucky bluegrass (*Poa pratensis* L.) and the bacterium had no effect on overseeded bermudagrass [*Cynodon dactylon* (L.) pers.] or perennial ryegrass (*Lolium perenne* L.) (Johnson, 1994).

Imaizumi and Fujimori (1997a) reported that once *Xantomonas campestris* was introduced through the plant wound (mowing), populations within the annual bluegrass plant increased by 1×10^8 colony forming units per gram (cfu g^{-1}) by 3 days after treatment (DAT), and 3.5×10^{10} cfu/g by 3 weeks after treatment (WAT). They also found that once the bacterial population reaches its peak of 1×10^{10} cfu/g (Imaizumi et al., 1998) in the plant, peak symptoms (blighted and dried leaves commonly associated with wilt diseases) were exhibited within one to two weeks. *Xanthomonas campestris* is translocated down the stem to the roots, then infecting the entire plant (Imaizumi and Fijimori, 1997b). Imaizumi et al. (1999) suggested that the mechanism by which the bacterium injures the annual bluegrass plant is occlusion of the xylem that results from the bacterial population within the plant reaching a maximum of 10^{10} cfu/g fresh weight. They also suggest that under conditions of high temperatures, Xanthomonads exude polysaccharides at an increased rate, thus contributing to the xylem occlusion that leads to plant death.

Movement and persistence

It is important to determine the fate of *Xanthomonas campestris* in turfgrass environments and to identify any potential for off-target effects. The movement and persistence of the bacterium in soils has been studied in recent years (Webber and Neal, 1992; Nishino et al., 1997; Nishino and Fujimori, 1998). Populations of *Xanthomonas campestris* decline rapidly once introduced to the soil. Webber and Neal (1992) determined that the population density of *Xanthomonas campestris* declined by 99.9% within 35 days of application. However, the bacterium was detected up to 1.4 meters away from its point of origin. Nishino et al. (1997) reported that isolate JT-P482 did not survive to a level of detection (1×10^3 cfu/gram of soil), and it had no significant impact on the microbial community in a natural soil environment. Nishino and Fujimori (1998) also showed that, while the bacterium persisted longer in the wound margins of creeping bentgrass than in the soil, populations dropped to below the detection limit within two months. Therefore off-target effects of *Xanthomonas campestris* may be limited.

Application procedures

Since the discovery of *Xanthomonas campestris* and its potential for biocontrol, much research has been directed toward the development of commercially practical application methods. As with any pesticide, a safe and effective rate must be determined, but because it is a living organism, many other biological factors must be considered. These factors include, but are not limited to, inoculum concentration; frequency, duration, and timing and method of applications; temperature, humidity, and soil moisture requirements for infection, and plant wounding.

Wounding

It is known that the pathogen needs a wound through which it enters the plant. The exact nature and means of creating the wound were studied by Webber et al. (1992) in the U.S., and by Imaizumi et al. (1997) in Japan. In growth chamber experiments, Webber et al. (1992) found that spraying of the suspension of 10^9 cfu ml^{-1} at the rate of 1,982 L ha^{-1} was more effective when done before mowing than after. Annual bluegrass control was increased 50% by spraying the suspension just before mowing, so that the fresh wound would be directly exposed to the bacterium.

Imaizumi et al. (1997) found it more effective to mow first, then spray. They also tested various types of wounds, including cutting of the oldest and youngest leaves, multiple leaves, seedhead stalk, stem, and roots, as well as rubbing one or several leaves with sandpaper (Imaizumi et al., 1998). The greatest number of injuries and, consequently, the most severe infection, resulted from cutting all of the stems.

Inoculum concentration

Many researchers have studied the effect of inoculum concentration on control of annual bluegrass. It is generally recommended for use at 10^9 cfu ml^{-1} at the rate of 946 L ha^{-1}. Imaizumi et al. (1997) reported that cell concentrations below 10^5 cfu ml^{-1} resulted in reduced efficacy (< 60% control), whereas higher concentrations than 10^9 cfu ml^{-1} did not significantly improve control.

Frequency and duration of applications

Xanthomonas campestris is an innundative biological control agent. It has been proven repeatedly that populations of the bacterium are not able to persist, much less proliferate to pathogenic levels, in the soil. Therefore, in order to cause infection, *Xanthomonas campestris* must be applied to the annual bluegrass repeatedly to achieve an acceptable level of control. Zhou and Neal (1995) found that the level of annual bluegrass control increased with both the number of consecutive weeks of application from one to four and application frequency from once to three times per week. Imaizumi et al. (1997) reported that repeated treatments with isolate JT-P482 resulted in annual bluegrass control levels of 85% or higher.

Climatic factors

One limitation to the use of fungal pathogens as biological control agents is that fungi often require specific environmental conditions such as temperature and humidity for the infection of plants. The influence of these factors on the potential infection of *Xanthomonas campestris* has been investigated.

Temperature. Activity of the bacterium requires temperatures within a specific range. Kalmowitz (1993) reported that the highest degree of biological control in growth chamber studies occurred at 26 C. Imaizumi et al. (1999) concluded that the most effective temperatures for biological control activity are from 30 to 35 C, and that the temperatures below 17 C and above 41 C provide usatisfactory levels of control (< 75%) (Imaizumi et al., 1999).

Humidity. Studies have been conducted to determine the effect of humidity on control of annual bluegrass by *Xanthomonas campestris*. A combination of inoculum concentration of 10^6 cfu ml^{-1}, low relative humidity, and high air temperature was effective for the control of annual bluegrass with *Xanthomonas campestris* (Nishino and Fujimori, 1996).

Soil moisture. Various researchers have studied the effect of water relations on development of vascular wilt symptoms. Webber and Neal (1992) found that, despite fresh and dry weight differences, the response to *Xanthomonas campestris* pv. *poannua* was more pronounced in plants maintained under non-limiting soil moisture than in those experiencing drought conditions. Kalmowitz (1993) reported that there was no significant effect of soil moisture on the activity of *Xanthomonas campestris* pv. *poannua*. However, in a study involving both creeping bentgrass and zoysiagrass (*Zoysia japonica* L.) putting greens, Imaizumi et al. (1999) suggested that low levels of annual bluegrass control in the bentgrass green may be attributed to a higher soil moisture level than what is typical for zoysiagrass green. The rapid wilting commonly associated with isolate *Xanthomonas campestris* may not occur under conditions of high soil moisture, perhaps because annual bluegrass is able to survive internal bacterial populations when soil moisture is high (Imaizumi et al. 1999).

Season. In the northeastern United States, the optimum temperature range for *Xanthomonas campestris* pv. *poannua* is reached only during the summer months, when it is applied (Webber and Neal, 1992; Zhou and Neal, 1995). However, Johnson (1996)

Season. In the northeastern United States, the optimum temperature range for *Xanthomonas campestris* pv. *poannua* is reached only during the summer months, when it is applied (Webber and Neal, 1992; Zhou and Neal, 1995). However, Johnson (1996) recommended winter applications for optimum control and Imaizumi et al. (1999) suggested that the most effective times to apply the bacterium are either late fall, targeting emergent seedlings, or early spring to control annual bluegrass before it produces seedheads.

Differential response of annual bluegrass biotypes

The perennial biotypes of annual bluegrass, *Poa annua* var. *reptans*, exhibit a greater tolerance to *Xanthomonas campestris* pv. *poannua* than the annual biotypes (Zhou and Neal 1995; Savage et al., 1995, personal communication). Inability of *Xanthomonas campestris* pv. *poannua* to satisfactorily control annual bluegrass in northern regions (where the perennial biotypes are predominant) has been attributed to this tolerance (Imaizumi et al. 1997). Imaizumi and Fujimori (1997) observed that the perennial biotype of annual bluegrass plants continued to grow and produced tillers, even when infected with bacterial populations that killed the annual biotype. They concluded that competition between plant growth and bacterial multiplication dictates the level of disease severity, and the differential response between biotypes could be attributed to the more aggressive nature of the perennial biotypes of annual bluegrass in that respect.

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CHAPTER II

EFFECTS OF VARIOUS MATERIALS ON THE GROWTH OF *XANTHOMONAS CAMPESTRIS* PV. *POANNUA*

Abstract

Xanthomonas campestris pv. *poannua* has potential as a biological control agent against annual bluegrass (*Poa annua* L.) in putting greens. The tolerance of *Xanthomonas campestris* to surfactants, growth regulators, and pesticides commonly used in putting green maintenance was examined. *Xanthomonas campestris* was grown on King's Media B agar amended with 0, 1, 10, 100, 1,000, and 10,000 ppm a.i. of each of five surfactants, two growth regulators, and four various pesticides. Colony survival 5 days after inoculation was recorded and a predicted LD_{50} value was calculated for each of the materials tested. Only Agri-dex was safe to the bacterium at recommended rates, among Agri-dex, Break-thru, Rely, Silwet L-77, and X-77. Trinexapac-ethyl and ECSS 1001 were safe to *Xanthomonas campestris* at recommended rates, and high rates of ECSS 1001 resulted in enlarged bacterial colonies. Only bifenthrin was toxic to *Xanthomonas campestris* at recommended rates, among ethofumesate, 2,4-D, bifenthrin, and chlorothalanil. Interactions of *Xanthomonas campestris* with these and other such compounds must be further examined with regard to bacterial survival and growth.

Introduction

Xanthomonas campestris pv. *poannua* has potential as a biological control agent against annual bluegrass (*Poa annua* L.) (Imaizumi et al., 1997; Johnson, 1994; Nishino et al., 1995) on putting greens. A variety of materials are often applied to putting greens for protection of the turfgrass from pests and to keep putting greens meeting the high standards of quality. While these materials must be proven safe for humans and for the environment, no data is available on how they will affect the survival of an imported bacterium.

Herbicides, growth regulators, fungicides, and insecticides can have lasting effects on plants in turfgrass environments. Applications of these materials timed closely to applications of the bacterium may cause them to come in contact with *Xanthomonas campestris*. The objectives of our experiments were to a) determine the effects of various materials common to putting green maintenance on colony survival of *Xanthomonas campestris*, and b) estimate LD_{50} values for each of the materials tested.

Materials and methods

Laboratory experiments were conducted with plant growth regulators, wetting agents, and various pesticides commonly used in the management of putting greens. Effects of these compounds on colony survival of *Xanthomonas campestris* in culture were examined.

The following compounds were added to King's Media B at concentrations of 0, 1, 10, 100, 1,000, and 10,000 ppm a.i.: trinexapac-ethyl [4-(cyclopropyl-α-hydroxy-methylene)-3,5- dioxo-cyclohexane-carboxylic acid ethyl ester] (Primo EC), ECSS-1001(an experimental plant growth regulator), Agri-dex (crop oil concentrate with a blend of 83% paraffin base petroleum oil, polyol fatty acid esters, and polyethoxylated derivatives), Break-thru (an organosilicone spreader adjuvant), Silwett L-77 (a nonionic silicone-polyether copolymer), X-77 (a non-ionic surfactant), Rely (a 99% blend of propoxylated and polyethylene glycols), 2,4-D (2,4-dichlorophenoxyacetic acid), ethofumesate [(±)-2-ethoxy-2, 3-dihydro-3, 3-dimethyl-5-benzofuranyl methanesulfonate], bifenthrin [2 methyl[1,1'-biphenyl]-3-yl] methyl 3-(2-chloro-3,3,3-trifluoro-1-propanyl]-2, 2-dimethylcyclopropanecarboxylate, and chlorothalanil (tetrachloroisophthalonitrile).

Media preparation

King's Media B was prepared in the laboratory by combining 38 g of Pseudomonas Agar F [Difco Laboratories (composed of 10 g Bacto Tryptone, 10 g Bacto Proteose Peptone No. 3, 1.5 g dipotassium phosphate, 1.5 g magnesium sulfate, and 15 g Bacto Agar)], 15 ml of glycerol (ACS certified, 99.9%), and 1L of deionized water per liter of medium. The mixture was autoclaved at 122 C and 114 kPa for 15 min. Amending materials were prepared and added to the agar prior to cooling in a laminar flow hood. Amended media was poured into Petri plates and allowed to cool.

Inoculation and incubation

Media plates were inoculated with *Xanthomonas campestris* by the addition of 0.1 ml of bacterial suspension containing approximately 5.0×10^3 colony forming units per milliliter (cfu ml^{-1}). Colonies were counted and recorded after 5 days of incubating at 28 C. Colony counts of the amended media treatments were converted to percent of the non-amended control. Non-linear regression was used to predict LD_{50} values (media concentration resulting in 50% colony survival) for each of the compounds tested.

Experimental design

Experimental design was a completely randomized block with three replications in each of two series. The data from the two series were pooled for each of the materials being tested. All data were subjected to analysis of variance to determine differences in treatment effects. Linear and nonlinear regression analyses were used to separate the means. F values with probabilities equal to or less than 0.05 were considered significant, and designated by an "*". F values of equal to or less than 0.01 were considered highly significant and designated by a "**".

Results

Toxicity of various materials to Xanthomonas campestris grown on amended King's Media B was presented in Table 2.1. Colony survival of *Xanthomonas campestris*

Table 2.1. Toxicity of various materials to *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

Material	LD_{50} (ppm)[a]	Use rates (ppm)[b]
Agri-dex	> 10,000	100 – 5,000
Break-thru	76	100 – 9,000
Rely	1,022	100 – 16,000
Silwet L-77	491	100 – 2,500
X-77	442	100 – 5,000
Trinexapac-ethyl	996	15 - 234
ECSS 1001	> 10,000	4
Ethofumesate	3,355	158 - 475
2,4-D	3,180	170
Bifenthrin	939	141 – 1,953

[a] Predictions based on mathematical models developed from actual observations.
[b] Spray solution concentrations, based on high and low labeled rates.

grown on amended King's Media B was not affected at 1ppm or 10 ppm concentrations of any of the materials tested, but was reduced by all materials at the rate of 10,000 ppm.

Agri-dex at 10,000 ppm reduced colony survival by 10%, but had no effect at lower rates (Figure 2.1). Break-thru, added to King's Media B at 100 and 1,000 ppm, reduced colony survival of *Xanthomonas campestris* by 70 and 100%, respectively (Figure 2.2). Rely concentrations up to 100 ppm did not reduce colony survival, but 1,000 and 10,000 ppm concentrations of Rely reduced colony survival by 44 and 100%, respectively (Figure 2.3). Silwet L-77 at concentrations of 100, 1,000, and 10,000 ppm reduced colony survival by 28, 70, and 100%, respectively (Figure 2.4).

The treatments of X-77 at 100, 1,000, and 10,000 ppm reduced colony survival by 18, 79, and 95%, respectively (Figure 2.5). Trinexapac-ethyl did not reduce colony survival at levels below 100 ppm, but prevented the survival of any colonies at levels above 1,000 ppm (Figure 2.6). Colony survival in agar amended with 1,000 ppm of ethofumesate or 2,4-D was 74%, while no colonies survived at the 10,000 ppm level of both herbicides (Figure 2.8 and Figure 2.9). The two highest levels of bifenthrin (1,000 and 10,000 ppm) reduced colony survival of *Xanthomonas campestris* by 55 and 100%, respectively (Figure 2.10).

Results from the chorothalanil-amended media study were inconsistent between the two series. In the first series of plates, colony survival was not reduced by chlorothalanil concentrations of 1,000 ppm or lower, but was completely (100%) reduced at 10,000 ppm (Figure 2.11). In the second series, however, only 43% of colonies survived 100 ppm and only 30% at 1,000 ppm.

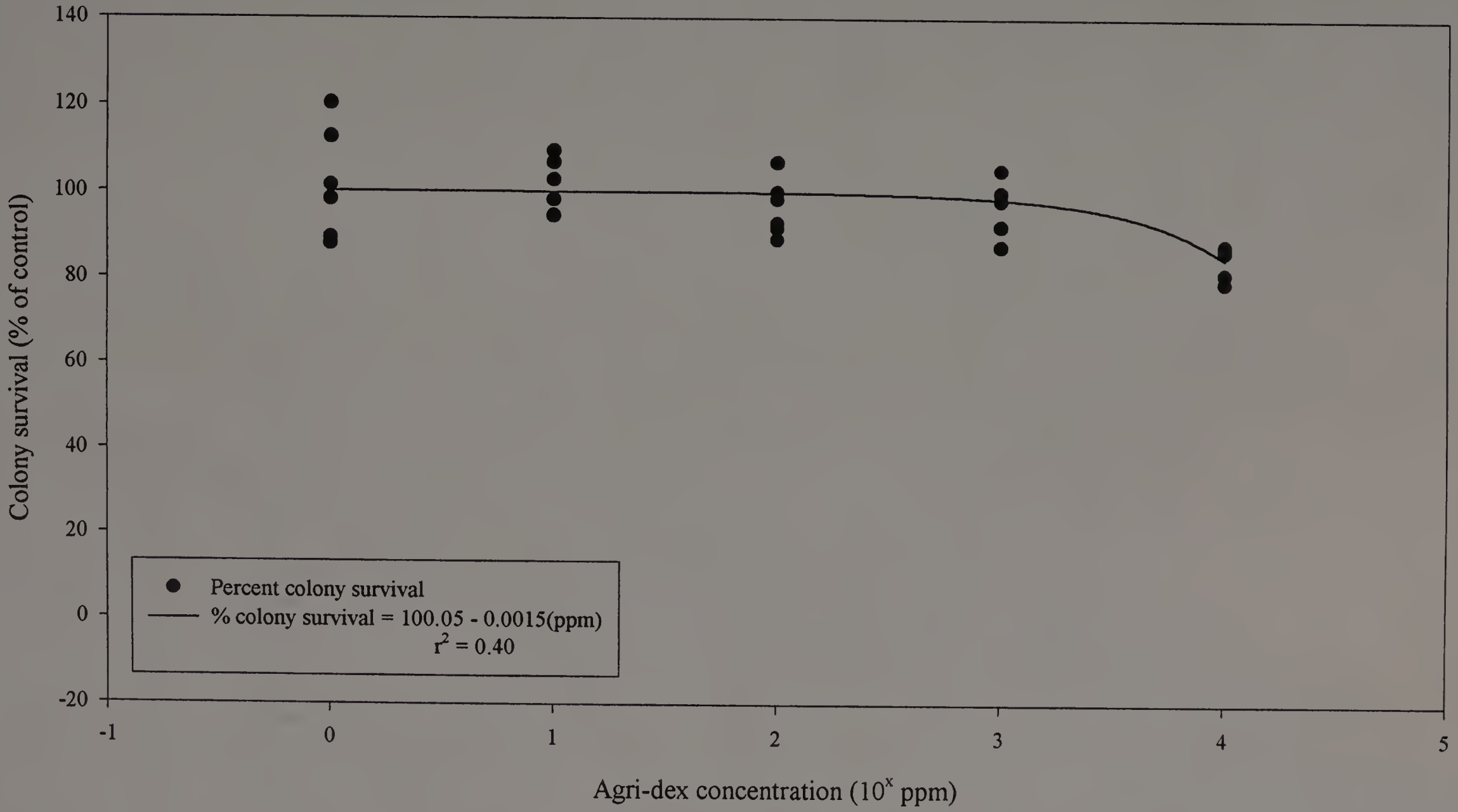

Figure 2.1. Regression line for the effect of Agri-dex on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

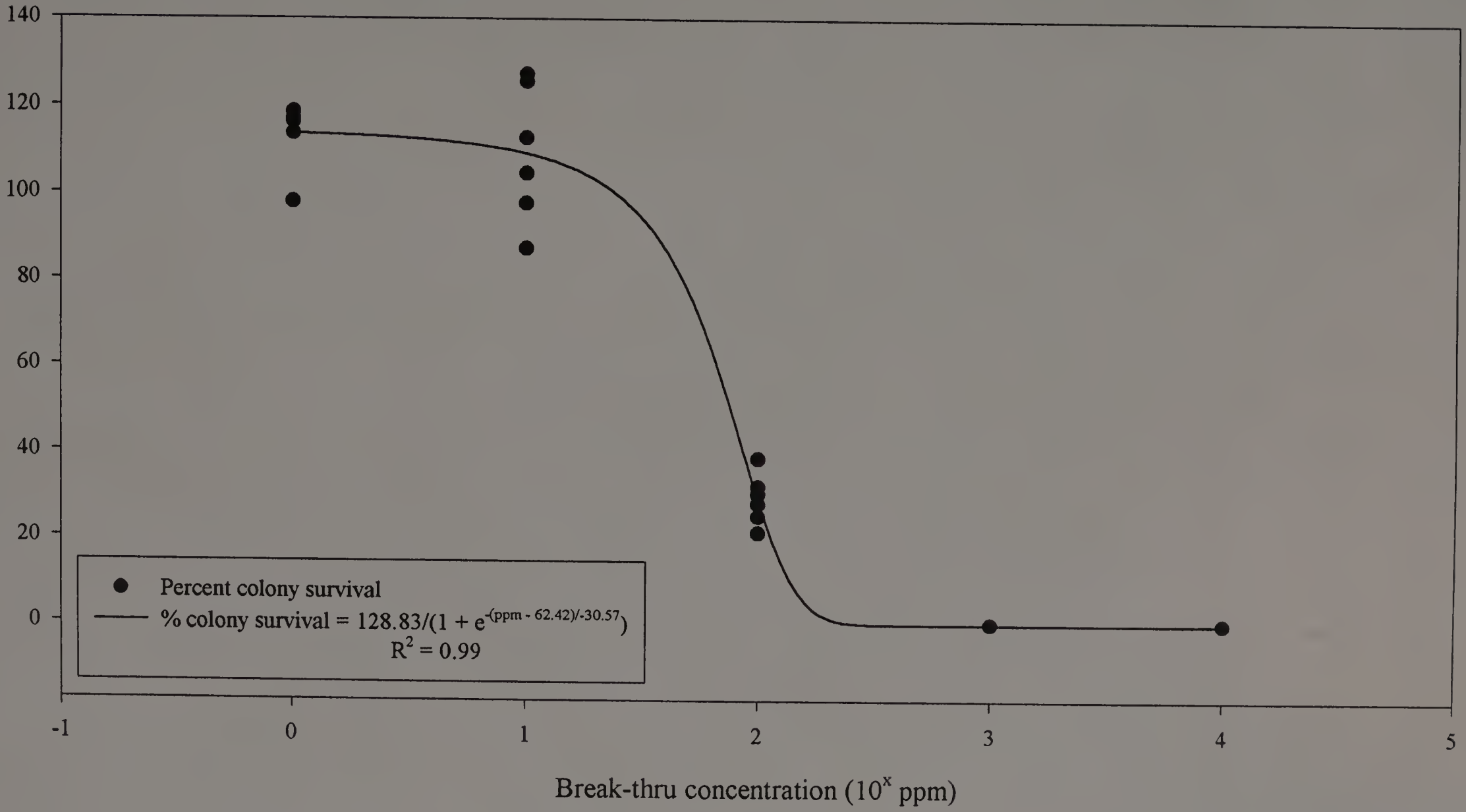

Figure 2.2. Regression curve for the effect of Break-thru on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

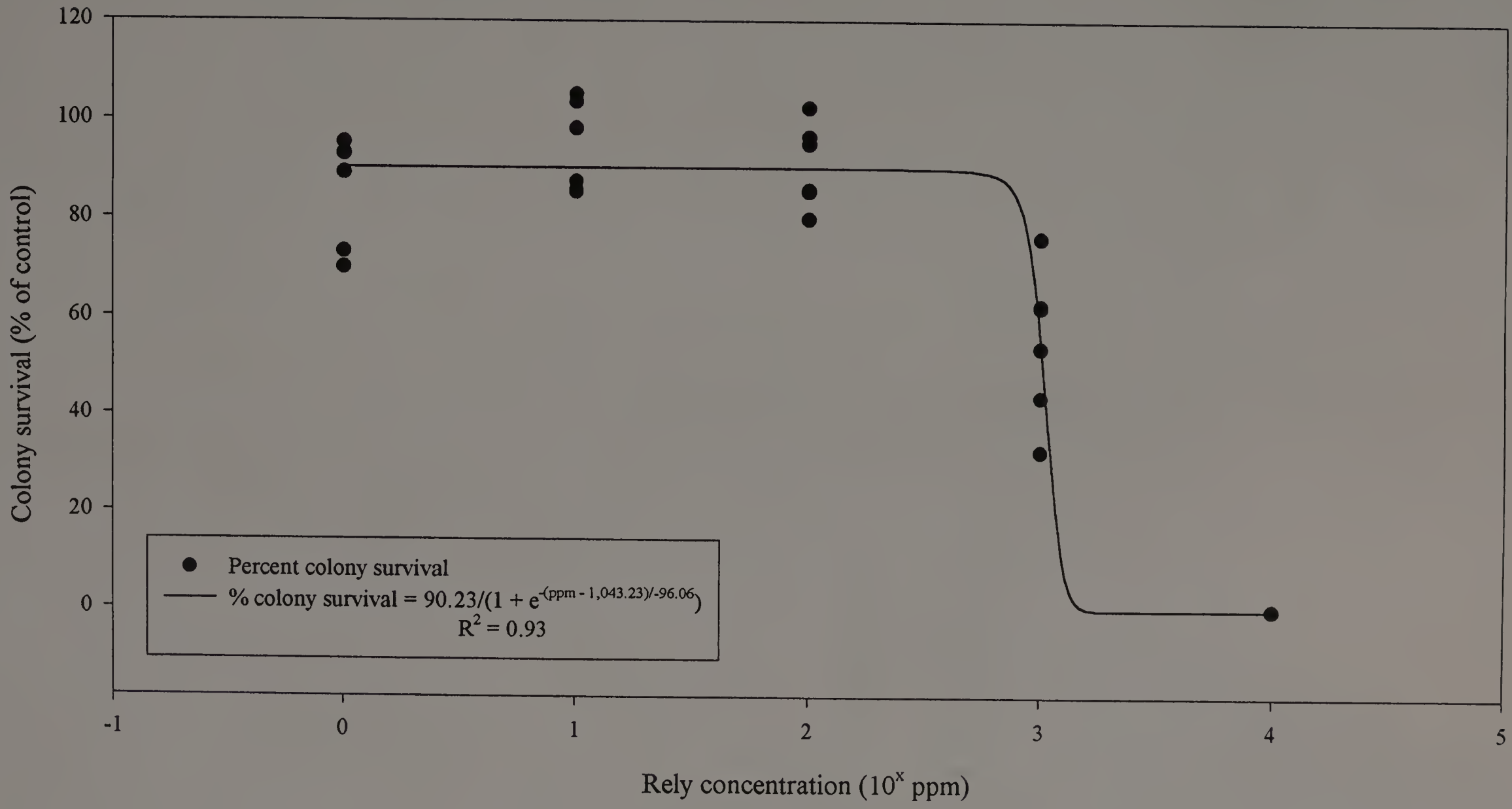

Figure 2.3. Regression curve for the effect of Rely on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

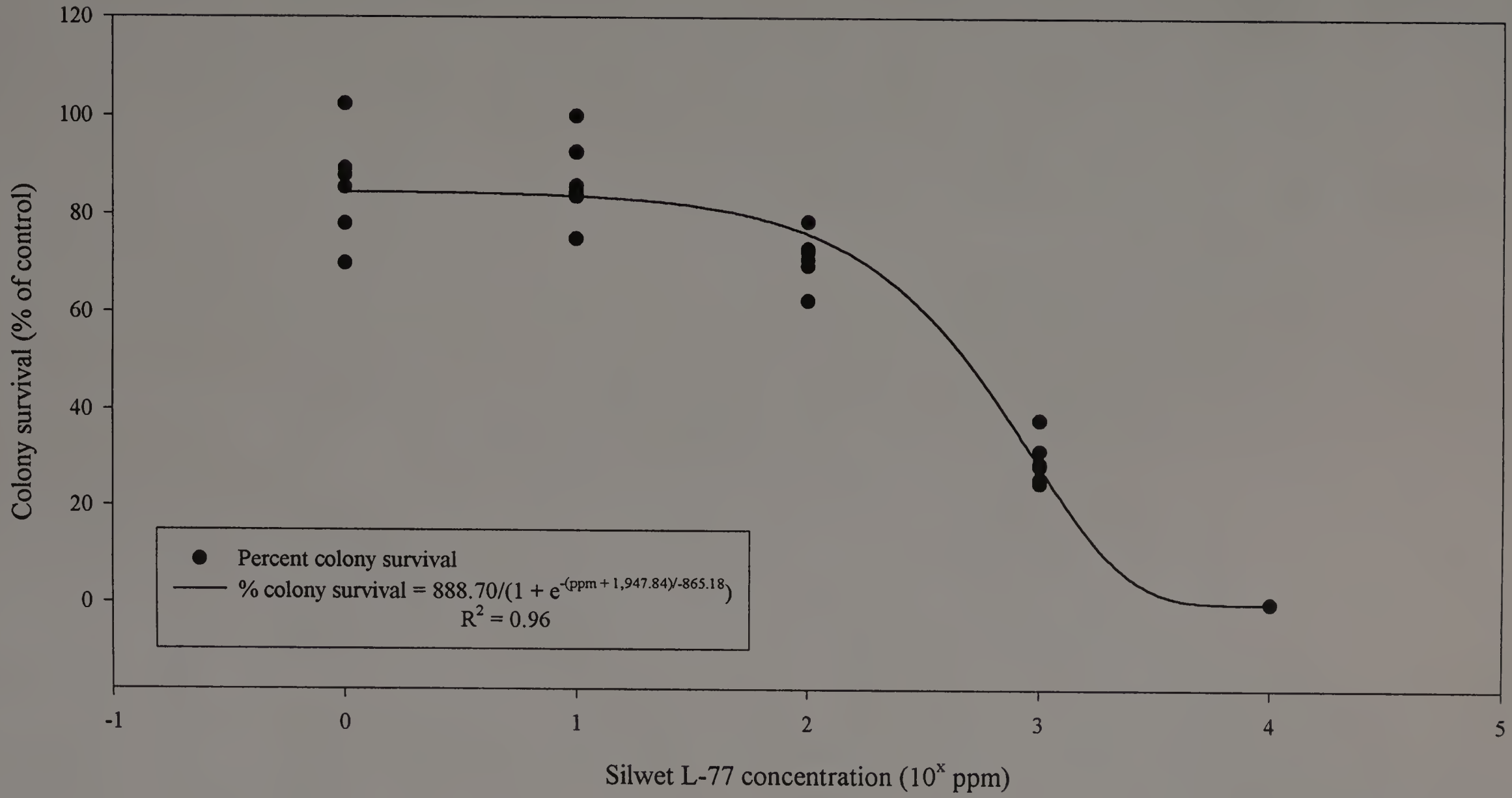

Figure 2.4. Regression curve for the effect of Silwet L-77 on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

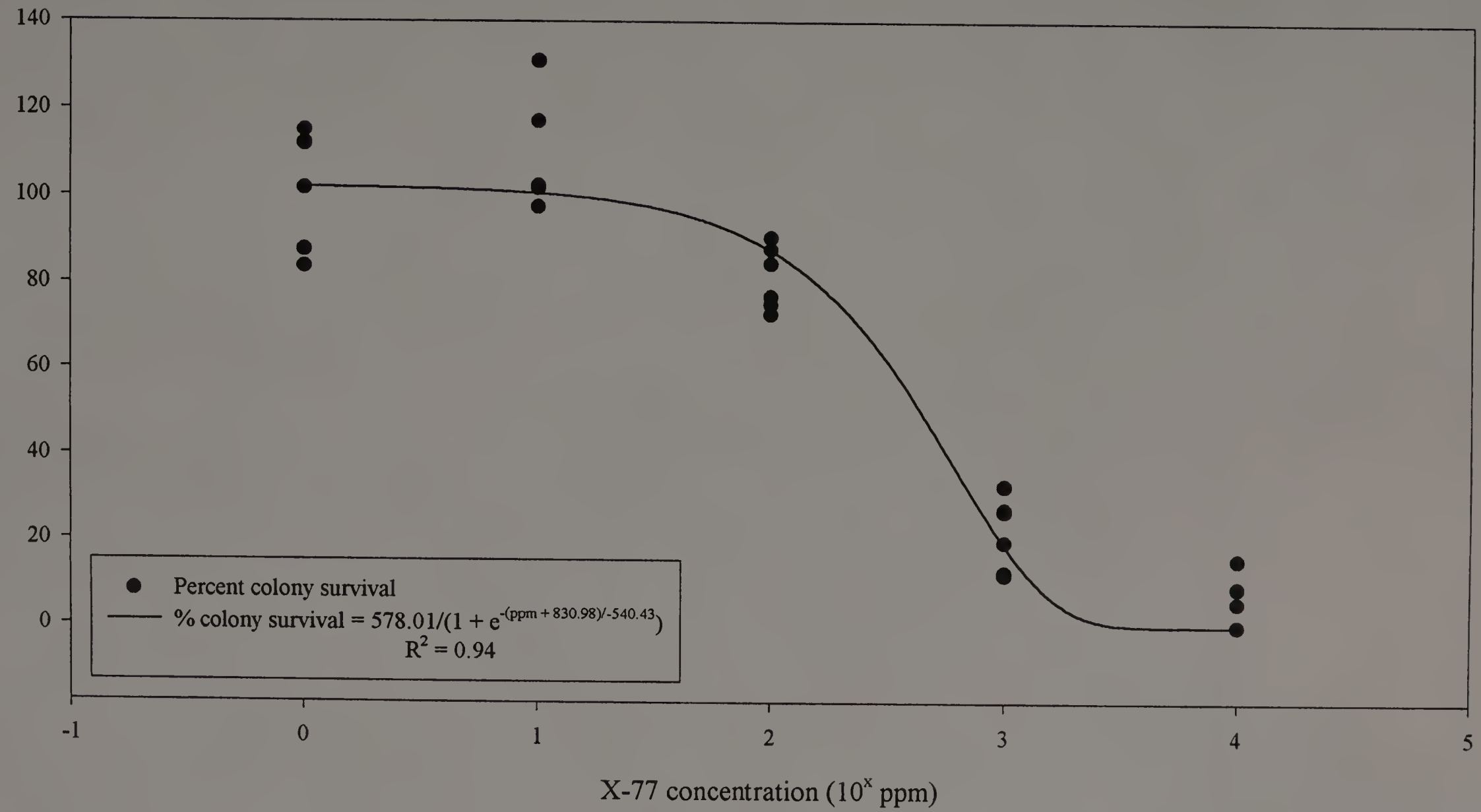

Figure 2.5. Regression curve for the effect of X-77 on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

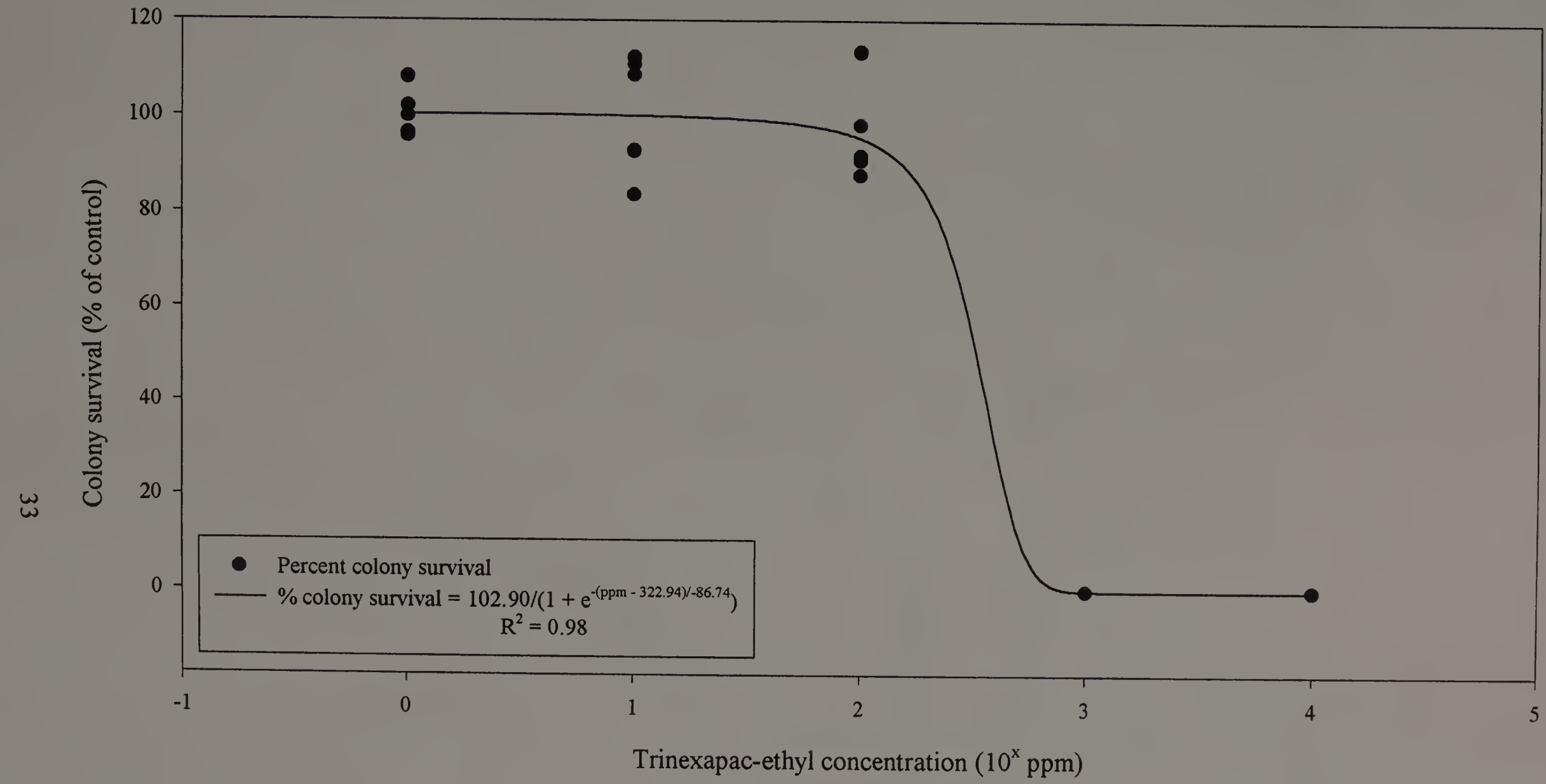

Figure 2.6. Regression curve for the effect of trinexapac-ethyl on colony survival of *Xanthomonas campestris* pv.*poannua* grown on amended King's Media B.

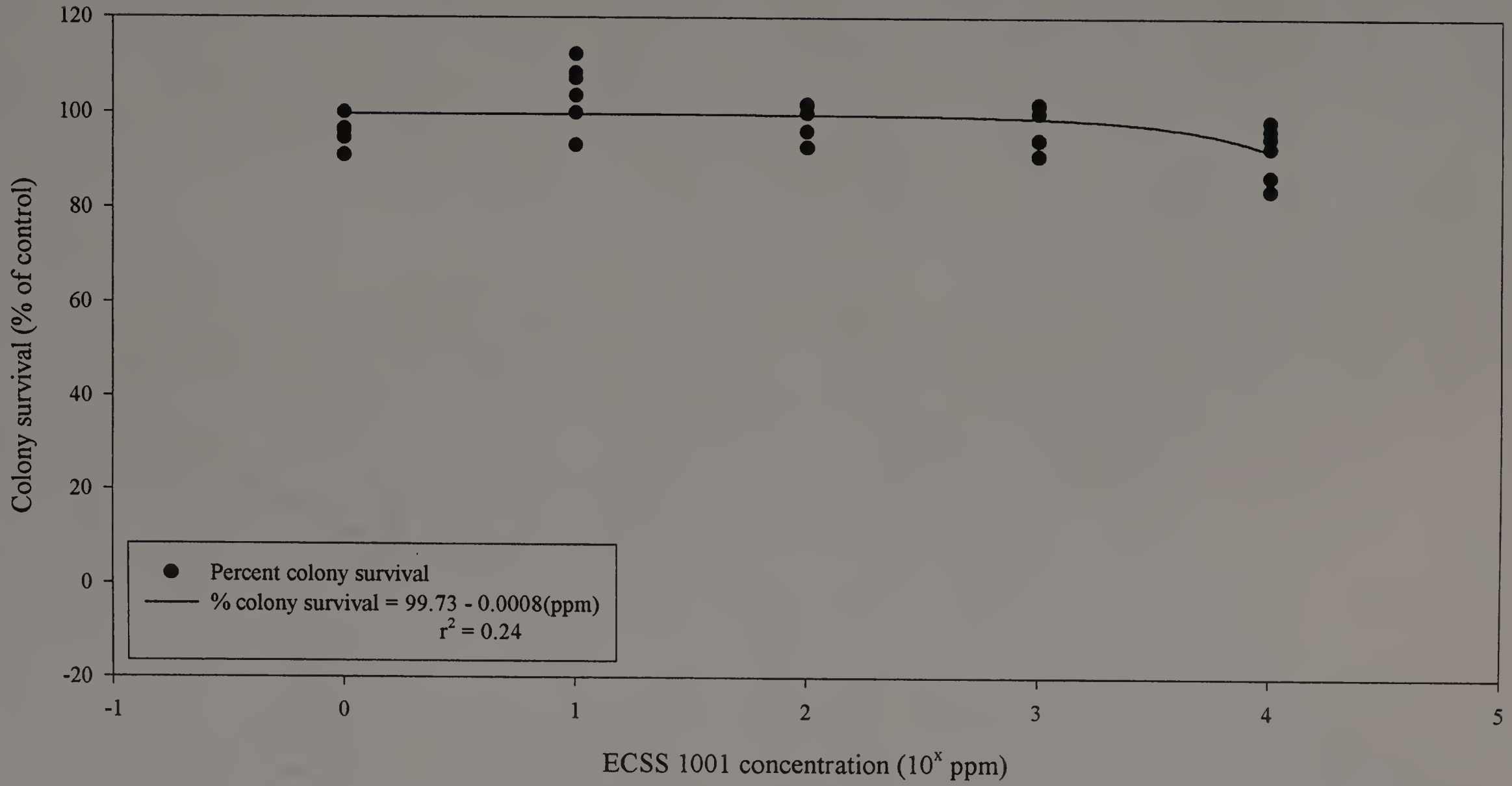

Figure 2.7. Regression line for the effect of ECSS 1001 on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

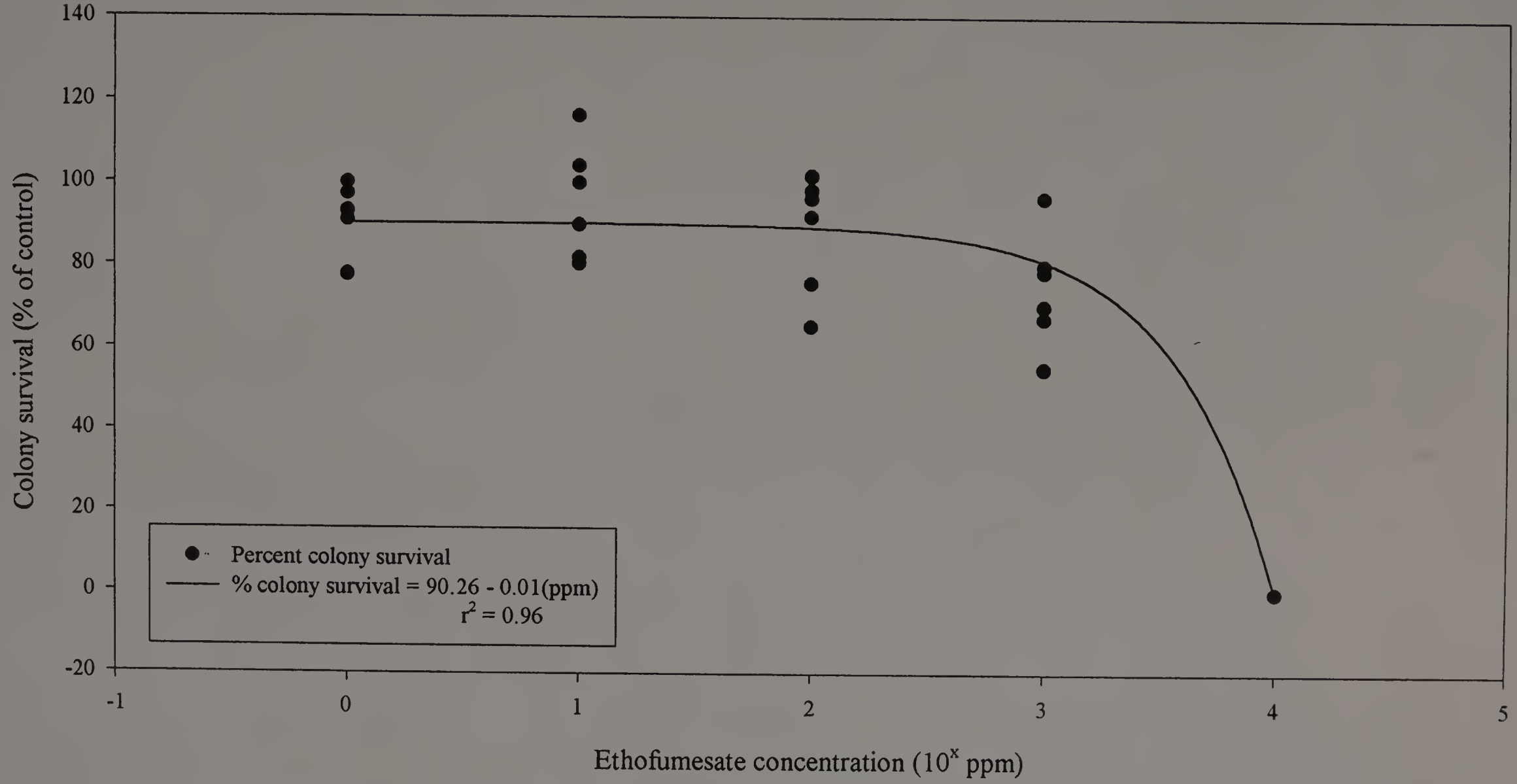

Figure 2.8. Regression line for the effect of ethofumesate on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

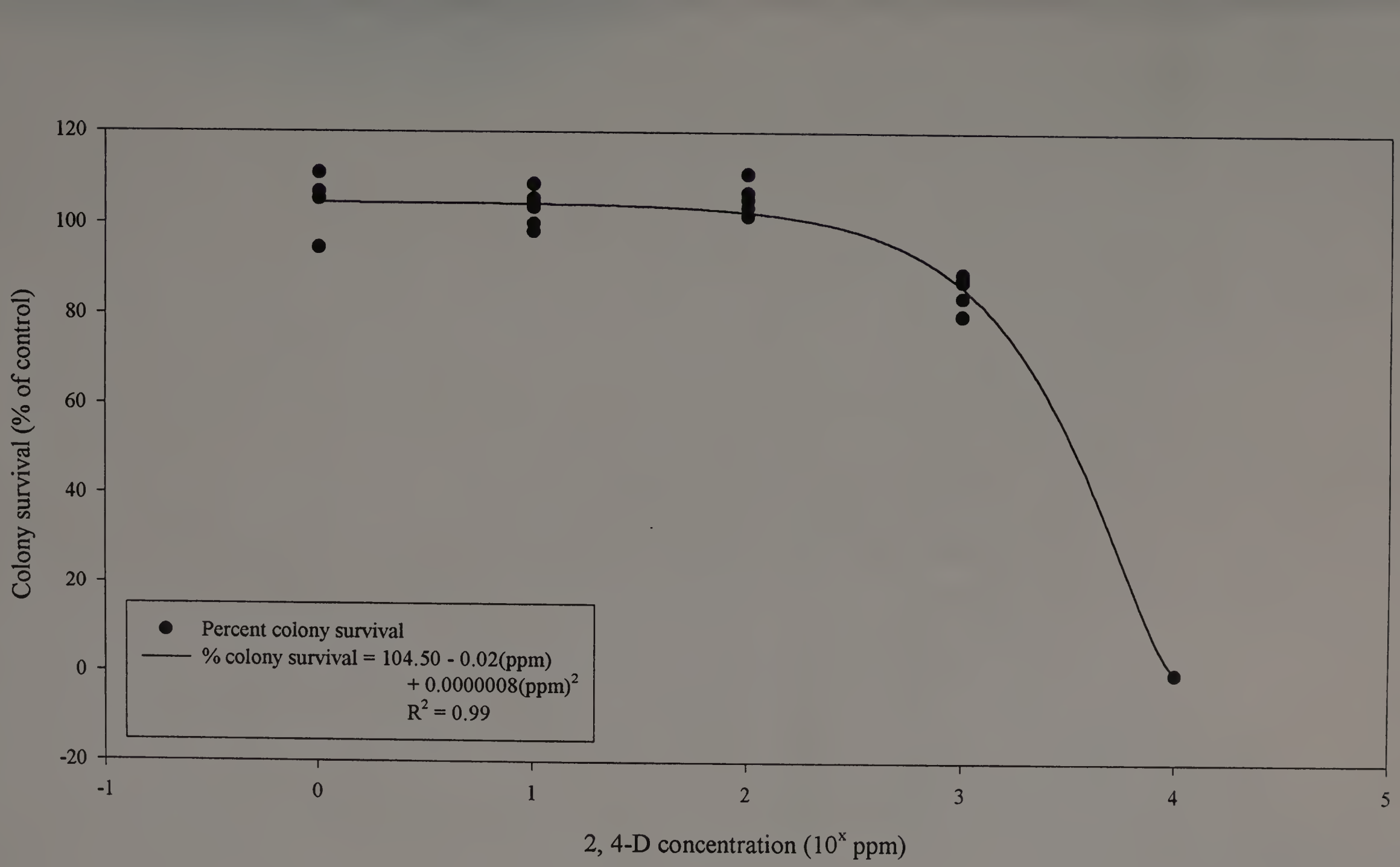

Figure 2.9. Regression curve for the effect of 2,4-D on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

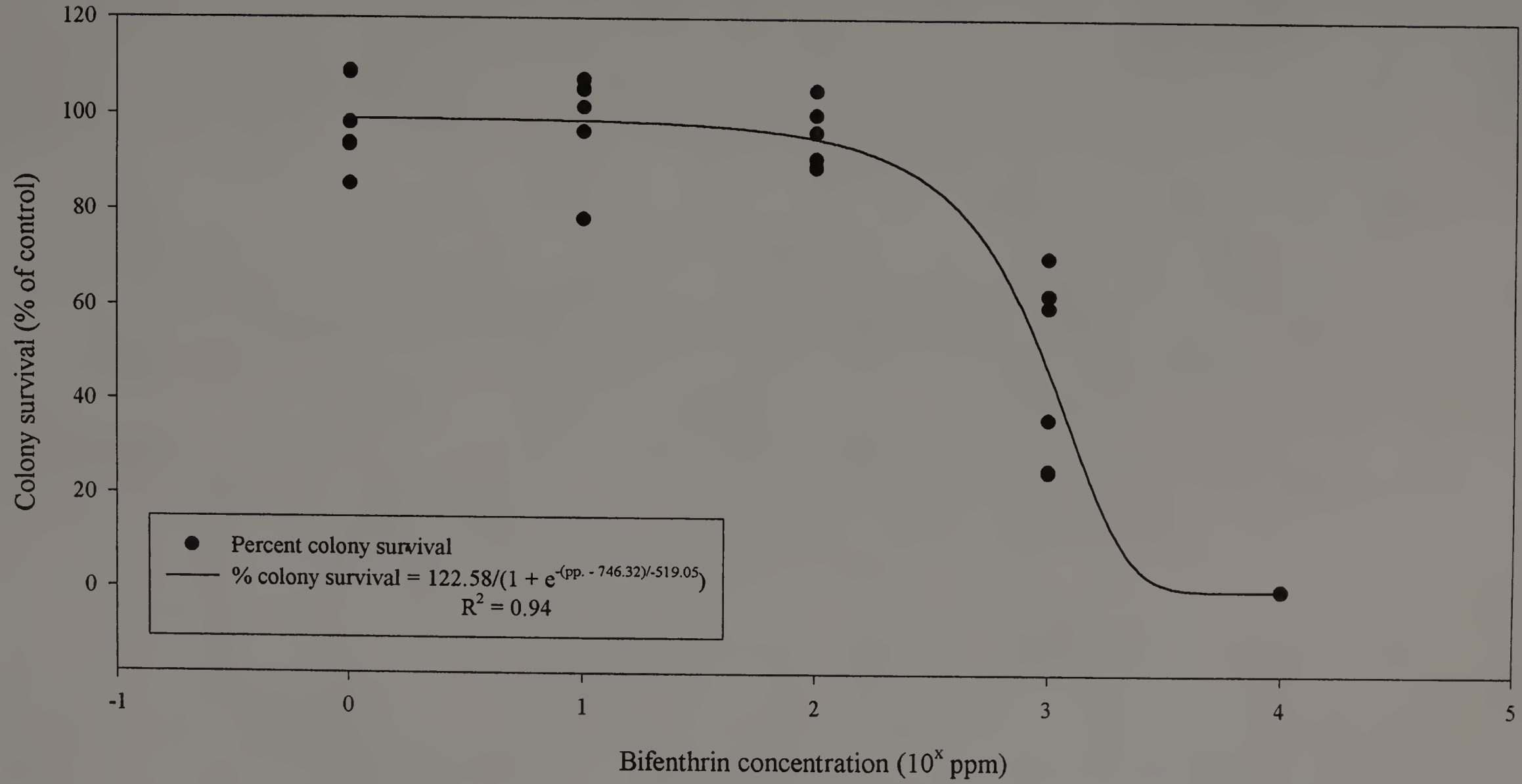

Figure 2.10. Regression curve for the effect of bifenthrin on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

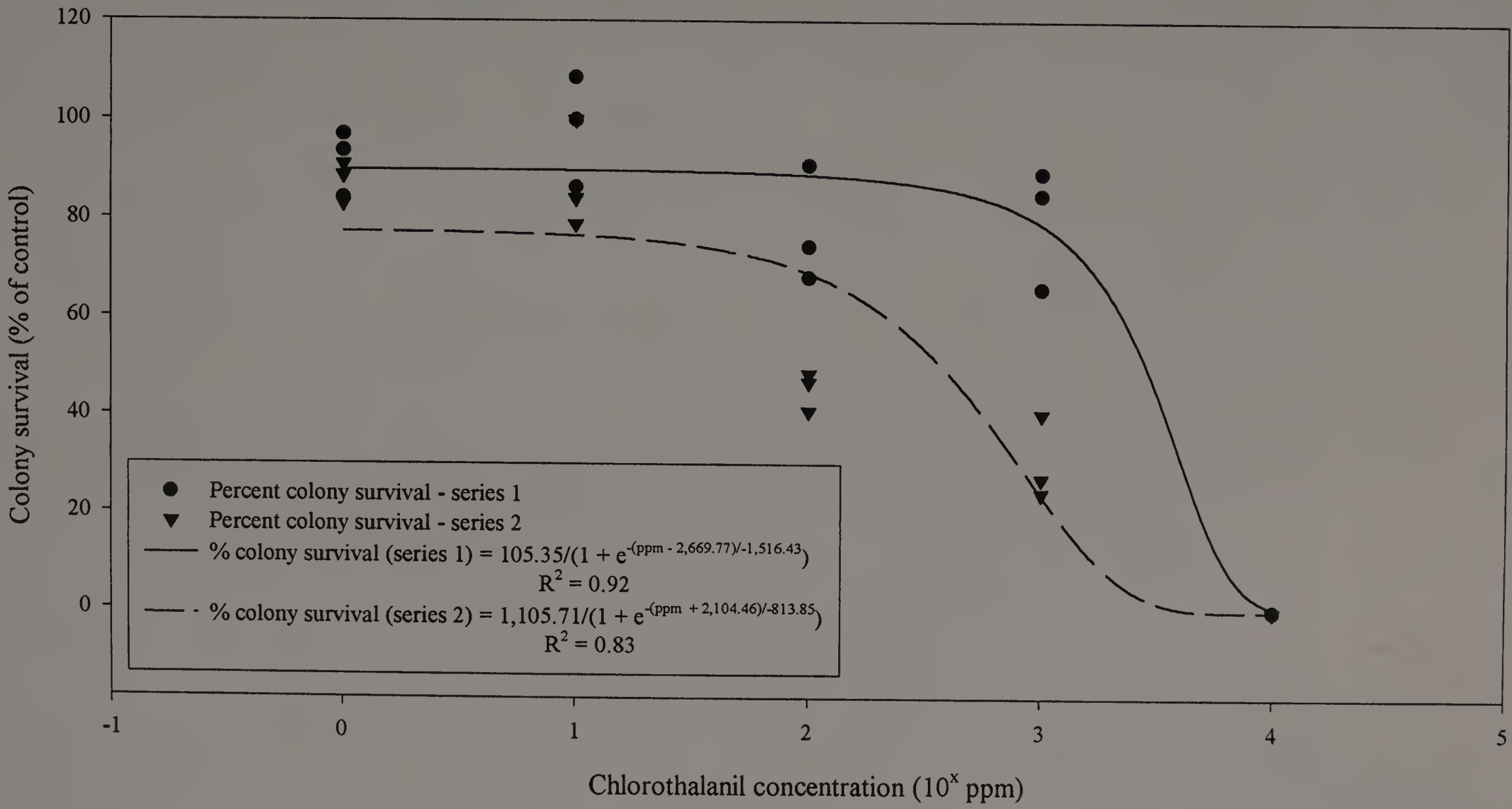

Figure 2.11. Regression curves for the effect of chlorothalanil on colony survival of *Xanthomonas campestris* pv. *poannua* grown on amended King's Media B.

Ninety-three percent of *Xanthomonas campestris* colonies survived on King's Media B containing 10,000 ppm of ECSS 1001 (Figure 2.7). In addition, colonies of *Xanthomonas campestris* grew several times larger on agar medium containing ECSS 1001 at 100 ppm and higher concentrations. The largest colony diameter was seen in the 10,000 ppm treatment. There were no apparent differences in cell morphology of these enlarged colonies as compared to cells from the control plates (under 400x magnification).

Discussion

The tolerance of *Xanthomonas campestris* to surfactants, growth regulators, and pesticides commonly used in putting green maintenance was examined. *Xanthomonas campestris* was unaffected by up to 10 ppm of all the materials tested. At concentrations of 100 to 10,000 ppm, the bacterium was variably sensitive to the materials tested. With the exception of Agri-dex (an oil adjuvant), each of the surfactants tested had LD_{50} values lower than their maximum use rates. Therefore, tank-mixing of surfactants and the bacterial suspension exposes the bacteria in the tank to concentrations of surfactants that correspond directly to use rates. Break-thru reduced colony survival of *Xanthomonas campestris* at rates as low as 100 ppm, which corresponds to a use rate of 0.01% (V/V). Rely became toxic to the bacterium at 1,000 ppm, or 0.1% (V/V). Both Silwet L-77 and X-77 were predicted to become toxic at levels higher than 500 ppm, or 0.05% (V/V). Tank-mixes of *Xanthomonas campestris* and high rates of Break-thru, Rely, Silwet L-77,

or X-77, might therefore be detrimental to the activity of the bacterium. Agri-dex, at recommended rates, was found to be safe to the growth of *Xanthomonas campestris*.

Trinexapac-ethyl, ethofumesate, 2,4-D, and bifenthrin were safe to *Xanthomonas campestris* at concentrations up to 100 ppm, while ECSS 1001 was safe up to 10,000 ppm. Predicted LD_{50} values of the same treatments were 996, 3,355, 3,180, 940 , and 62,163 ppm, respectively. Only bifenthrin is recommended at use rates that may be toxic to *Xanthomonas campestris.*

Regardless of whether or not materials are tank-mixed with the bacterium, the possibility of exposing the bacterium to materials that may be applied to putting greens exists. Interactions of *Xanthomonas campestris* with such materials must be further examined with regard to bacterial survival and growth.

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CHAPTER III

EFFECTS OF SURFACTANTS ON THE ACTIVITY OF *XANTHOMONAS CAMPESTRIS* PV. *POANNUA* IN ANNUAL BLUEGRASS SUPPRESSION

Abstract

Xanthomonas campestris pv. *poannua* has potential as a biological control agent against annual bluegrass (*Poa annua* L.) in putting greens. Growth room and field experiments were conducted to determine the effects of various surfactants on the activity of *Xanthomonas campestris* in the suppression of annual and perennial biotypes of annual bluegrass. Effects of surfactants and the bacterium on creeping bentgrass (*Agrostis palustris* Huds.) were also examined. *Xanthomonas campestris* at 10^7 cfu ml^{-1} suppressed both biotypes of annual bluegrass by 100% in the growth room, yet did not suppress annual bluegrass in field trials. Agri-dex accelerated infection of the annual biotype of annual bluegrass by 1 WAT, but did not affect annual bluegrass suppression by *Xanthomonas campestris*. Rely and Rewet did not affect bacterial activity or creeping bentgrass. Silwett L-77, X-77, and high rates of Break-thru (0.6 and 0.9% V/V) reduced activity of *Xanthomonas campestris* by 23, 35, and 87%, respectively. Break-thru at 0.9% (V/V) injured creeping bentgrass. It is possible that adequate internal *Xanthomonas campestris* populations are not established in annual bluegrass under the exposure to toxic levels of surfactants.

Introduction

Xanthomonas campestris pv. *poannua* causes vascular wilt of annual bluegrass *Poa annua* L.). A wound, such as that caused by mowing, is required for the bacterium to infect the plant. Imaizumi et al. (1997) determined that the most effective type of wound for infection by *Xanthomonas campestris* is the one resulting in the highest number and severity of injuries to the annual bluegrass plant - made by cutting through all of the stems. Daily mowing of putting greens limits the amount of growth between mowings and therefore limits the likelihood of wounding several stems on each annual bluegrass plant. Since cutting through all of the stems of annual bluegrass plants is not practical in a putting green environment, alternative methods of improving infection must be explored.

Surfactants can improve the emulsifying, dispersing, spreading, or other properties of a liquid to reduce the surface tension of water droplets containing herbicide or other materials and therefore, increase contact with the leaf surface. The addition of Agri-dex at 1.0% (V/V) to rimsulfuron at 9.0 g ha^{-1} increased control of lambsguarters (*Chenopodium album* L.) by 70% 3 weeks after treatment (Mitra et al., 1998).

If more bacteria come into contact with the surface of the wound, more cells may gain entry into the plant, causing a higher rate of infection. Increasing the rate of infection of the bacterial applications might enhance development of the vascular wilt disease in annual bluegrass and ultimately accelerate suppression of annual bluegrass.

The objectives of our experiments were to a) determine the effects of various surfactants on the activity of *Xanthomonas campestris* in suppressing growth or seedhead production of annual bluegrass, and b) to determine the effects of *Xanthomonas campestris* and surfactants on the quality of creeping bentgrass on a putting green.

Materials and methods

Growth room experiments

Two experiments were conducted in a growth room. Annual bluegrass and creeping bentgrass were grown side by side in 11.4 cm diam. pots. Seeds of an annual biotype of annual bluegrass (*Poa annua* var. *annua*) and 'G2' creeping bentgrass were sown at 152.5 kg ha^{-1} and 76.2 kg ha^{-1}, respectively. Plugs of a perennial biotype of annual bluegrass (*Poa annua* var. *reptans*) measuring 5.1 cm in diam. by 5.1 cm deep were collected from a putting green at the Turf Research Center in South Deerfield, MA. The plugs were transplanted into 10.2 cm diam. pots. The soil was a Hadley fine sandy loam (Typic Udifluvents) containing 3.5% organic matter, with a pH of 6.5. The soil was autoclaved prior to seeding to eliminate the potential for interference by organisms inhabiting the soil. The growth room had an average temperature of 24 C (± 2 C) and relative humidity of 32% (± 3%). Fluorescent lighting was used in a 16 h cycle. The plants were watered and fertilized as needed to avoid water or nutritional stress, and clipped twice weekly to a height of 2.5 cm.

Experiment No. 1.

One oil adjuvant [Agri-dex (crop oil concentrate with a blend of 83% paraffin base petroleum oil, polyol fatty acid esters, and polyethoxylated derivatives)] and three surfactants [Break-thru (an organosilicone spreader adjuvant), Silwett L-77 (a non-ionic silicone-polyether copolymer) and X-77 (a nonionic surfactant)] were used with *Xanthomonas campestris*. Agri-dex, Break-thru, Silwet L-77 and X-77 were added to deionized water at rates of 0.5%, 0.75%, 0.25%, and 0.5% (V/V), respectively. A prepared liquid culture of *Xanthomonas campestris* containing 10^9 colony forming units per milliliter (cfu ml^{-1}) was added to each solution at 10 ml L^{-1}. Additional treatments included the bacterium alone and an untreated control. The treatments were applied to both annual and perennial types of annual bluegrass and creeping bentgrass at 946 L ha^{-1}, using a hand pump sprayer. The plants were clipped with clean grass shears within five minutes of each treatment application. Five applications were made at one-week intervals. All treatments were replicated four times in a completely randomized block design.

Experiment No. 2.

In this study, the effects of various rates of three surfactants on the activity of *Xanthomonas campestris* in controlling annual bluegrass were examined. The surfactants were Rely [99% (blend of propoxylated and polyethylene glycol)], Rewet [10% (polyalkalene glycol)], and Break-thru]. The bacterium was applied in a suspension containing 10^9 cfu ml^{-1} at 9.46 L ha^{-1}. Additional treatments included the bacterium alone

and an untreated control. The treatments were applied to both annual and perennial biotypes of annual bluegrass and creeping bentgrass at 946 L ha^{-1}, using a hand pump sprayer. The plants were clipped with a clean grass shear within five minutes of each treatment application. Five applications were made at one-week intervals. All treatments were replicated four times in a completely randomized block design.

Visual ratings

Quality of annual bluegrass and creeping bentgrass, and seedhead production by annual bluegrass were evaluated by visual ratings. Ratings were made weekly throughout the course of the experiment. Annual bluegrass control was determined at 3 , 4 and 7 weeks after the first treatment (WAT). Visual ratings of both annual and perennial types of annual bluegrass, and creeping bentgrass were estimated based on a 1 to 9 scale, where 1 = dead grass and 9 = healthy grass. Seedhead production of the perennial type annual bluegrass was estimated based on a scale of 0 to 100%, where 0 = no seedheads were produced and 100 = all shoots produced seedheads.

Clipping fresh weights

All plants were clipped twice a week, once immediately following treatment and once three days later, at which times clipping fresh weights were recorded. The clipping weight of each treatment was converted to a percent of the clipping weight of the untreated control.

Experimental design

Both experiments were laid out in a completely randomized block design. Analysis of variance (ANOVA) was used to determine differences in treatment effects. Error terms were calculated using the expected mean squares (Damon and Harvey, 1987). Hypothesis tests were performed with the appropriate error terms by using the general linear model program of SAS (SAS Institute, 1995). F values with probabilities equal to or less than 0.05 were considered significant, and designated by an "*". Those with F values of equal to or less than 0.01 were considered highly significant and designated by a "**". The means were separated using Duncan's New Multiple Range Test (P = 0.05).

Field experiments

Two field experiments were conducted at the Turf Research Center in South Deerfield. The soil was composed of sand and native Winooski soil, with a pH of 6.0. Plots were laid out on a two-year old putting green with either 'Penncross' or 'Providence' creeping bentgrass. The area was heavily infested with perennial type of annual bluegrass. The putting green was mowed three times a week at a height of 6.35 mm, including once for treatment applications. The putting green was maintained according to a regular program, including normal irrigation practices and disease management practices that excluded the use of systemic fungicides. Fertility was monitored closely and nitrogen was applied every two weeks at 12.7 kg ha^{-1}.

In both experiments, five weekly applications were made during the month of May, 2000. Applications were carried out after 4:30 pm to minimize the effects of UV

light on *Xanthomonas campestris* activity. Surfactants were added to water in separate bottles in the laboratory and the bacterium was added just before spraying. Treatments were applied using an 11.4 L compressed air sprayer at a spray volume of 946 L ha^{-1}. The treated plots were mowed within five minutes of spraying to protect the bacterium against desication. The mowing was done with a Jacobsen walk-behind greensmower.

Experiment No.1.

Agri-dex, Break-thru, Silwett L-77, and X-77 were used with *Xanthomonas campestris.* These materials were added to deionized water at 0.5%, 0.75%, 0.25%, and 0.5% (V/V), respectively. A prepared liquid culture of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was added to each solution at the recommended rate of 10 ml L^{-1}. Additional treatments included the bacterium alone and an untreated check. The plots were 1.22 by 3.05 m, arranged in a non-randomized (due to the mowing requirement) complete block design with six replications.

Experiment No.2.

The effect of three rates of three surfactants on the activity of *Xanthomonas campestris* in annual bluegrass control was examined. Rely was applied at 6.44, 12.88, and 19.32 L ha^{-1}. Rewet was applied at 16.10, 22.54, and 32.20 L ha^{-1}. Break-thru was applied at 0.3, 0.6, and 0.9% (V/V). A prepared liquid culture of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was tank mixed with each of the treatments at 10 ml L^{-1} and treatments were applied at 946 L ha^{-1}. Additional treatments included the

bacterium alone and an untreated control. The plots were 0.92 by 3.05 m, arranged in a non-randomized (due to the mowing requirement) complete block design with six replications.

Visual ratings

Visual ratings of annual bluegrass cover, seedhead production, and quality were recorded periodically beginning at the onset of the experiment and continuing through 14 WAT. Creeping bentgrass quality was visually rated periodically from 3 to 14 WAT. Annual bluegrass cover was estimated as percentage of plot area occupied by annual bluegrass. Ratings of annual bluegrass cover made after the first rating were converted to change in annual bluegrass cover by subtracting the initial week's rating. Seedhead production was estimated visually on a scale of 0 to 100%, where 0 = none of the annual bluegrass shoots had seedheads and 100% = all of the annual bluegrass shoots had seedheads. Annual bluegrass quality ratings were made on a scale from 1 to 9, where 1 = dead and 9 = healthy. Creeping bentgrass quality ratings were made on a 1 to 9 scale, where 1 = dead grass and 9 = a healthy, dense, uniform stand of turfgrass. Ratings were made with consistency regarding time of day and time after mowing.

Experimental design

All data were subjected to analysis of variance (ANOVA) to determine differences in treatment effects. Error terms were calculated using the expected mean squares (Damon and Harvey, 1987). Hypothesis tests were performed with the

appropriate error terms by using the general linear model program of SAS (SAS Institute, 1995). F values with probabilities equal to or less than 0.05 were considered significant, and designated by an "*". Those with F values of equal to or less than 0.01 were considered highly significant and designated by a "**". The means were separated using Duncan's New Multiple Range Test (P = 0.05) (Damon and Harvey, 1987).

Results

Growth room experiments

Xanthomonas campestris applied at 10^7 cfu ml^{-1} suppressed annual and perennial biotypes of annual bluegrass by 96% (Table 3.1) and 92% (Table 3.2) 7 WAT (Figure 3.1). Beginning at 2 WAT, annual bluegrass plants treated with the bacterium exhibited blighted and dried leaves. Bacterial streaming was visible from cut stems of infected annual bluegrass plants under 40 x magnification. Clipping fresh weight and quality of both biotypes of annual bluegrass declined shortly after symptoms became visible (Tables 3.3 and 3.4; Figure 3.2). Neither quality nor clipping fresh weight of creeping bentgrass was affected by *Xanthomonas campestris* (Tables 3.5 and 3.6; Figure 3.2). Seedhead production was not affected by the bacterium alone or in combination with surfactants (Table 3.7).

Experiment No. 1.

Tank mixing of Agri-dex and the bacterial suspension at 0.5 % (V/V) reduced the quality and clipping fresh weight of the annual biotype of annual bluegrass by 40%

Table 3.1. Suppression of an annual biotype of annual bluegrass by *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various surfactants.

		Percent weed suppression		
		WAT[a]		
Treatment	% (V/V)	3	4	7
Untreated Control		0.00 c	0.00 b	0.00 b
Xanthomonas campestris[b] alone	1.0	87.50 a	90.00 a	96.25 a
Agri-dex	0.5	91.25 a	93.75 a	98.75 a
+				
Xanthomonas campestris	1.0			
Break-thru	0.75	67.50 b	95.00 a	97.50 a
+				
Xanthomonas campestris	1.0			
Silwet L-77	0.25	80.00 ab	92.50 a	93.75 a
+				
Xanthomonas campestris	1.0			
X-77	0.5	65.00 b	90.00 a	92.50a
+				
Xanthomonas campestris	1.0			
F-value		**	**	**

[a] WAT = weeks after first application.

[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.2. Suppression of a perennial biotype of annual bluegrass by *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various surfactants.

		Percent weed suppression		
		WAT[a]		
Treatment	% (V/V)	3	4	7
Untreated Control		0.00 b	0.00 b	0.00 c
Xanthomonas campestris[b] alone	1.0	25.00 b	32.50 ab	92.50 a
Agri-dex	0.5	27.50 b	52.50 ab	100.00 a
+				
Xanthomonas campestris	1.0			
Break-thru	0.75	82.50 a	78.75 a	98.75 a
+				
Xanthomonas campestris	1.0			
Silwet L-77	0.25	2.50 b	10.00 ab	76.25 ab
+				
Xanthomonas campestris	1.0			
X-77	0.5	0.00 b	26.50 ab	57.50 b
+				
Xanthomonas campestris	1.0			
F-value		**	*	**

[a] WAT = weeks after first application.

[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

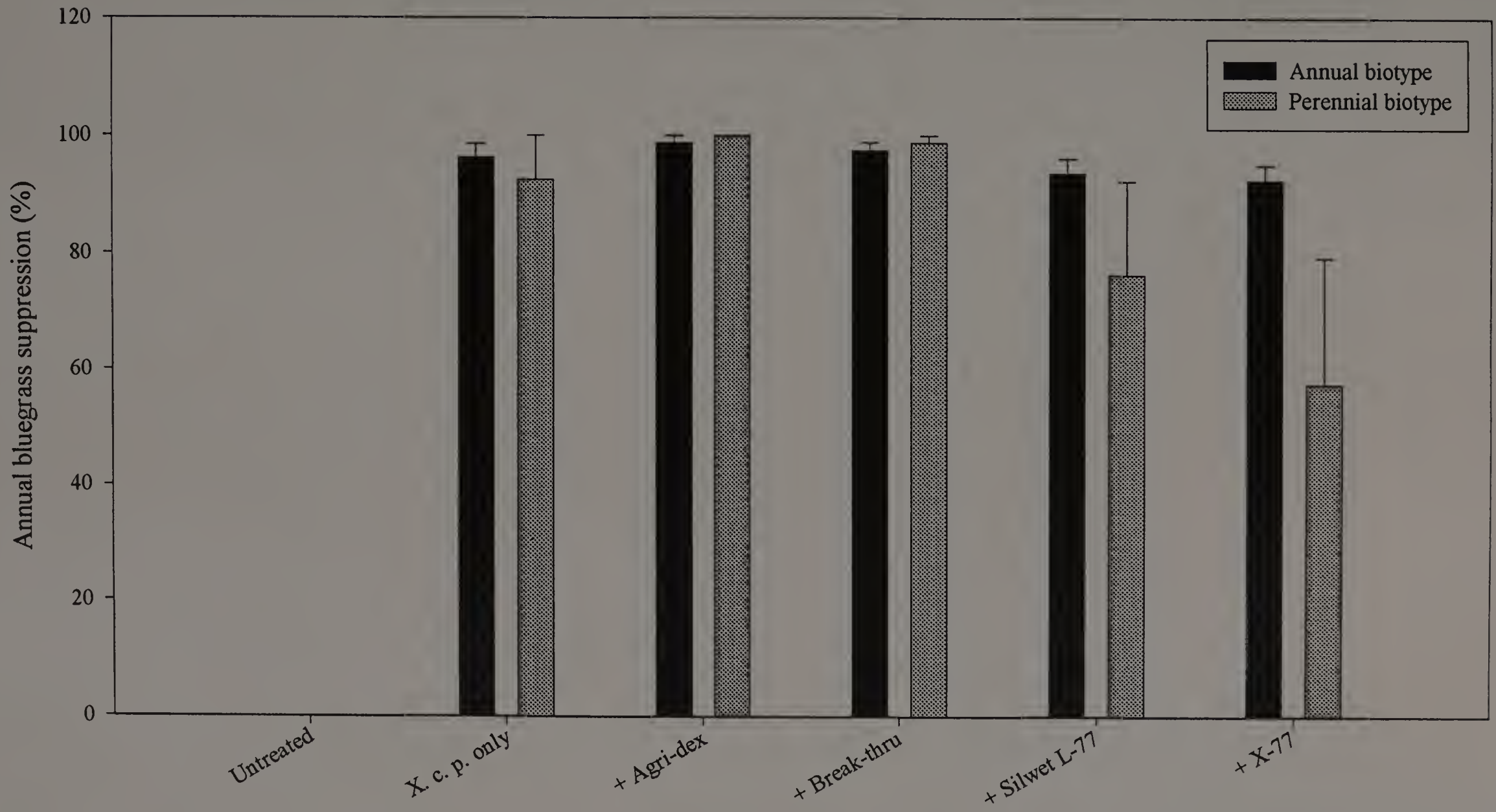

Figure 3.1. Suppression of two biotypes of annual bluegrass with *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various surfactants 7 WAT.

Table 3.3. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of an annual biotype of annual bluegrass under controlled conditions as influenced by various surfactants.

Treatment	% (V/V)	Clipping fresh weight (% of untreated control) WAT[a] 0	1	2	3	4
Xanthomonas campestris[b] alone	1.0	122.61	115.20	52.87 a	10.53 c	3.41
Agri-dex + *Xanthomonas campestris*	0.5 + 1.0	122.66	98.14	24.25 b	7.53 c	5.04
Break-thru + *Xanthomonas campestris*	0.75 + 1.0	137.69	115.03	53.94 a	20.17 bc	3.41
Silwet L-77 + *Xanthomonas campestris*	0.25 + 1.0	125.88	104.60	69.48 a	26.44 b	9.67
X-77 + *Xanthomonas campestris*	0.5 + 1.0	135.25	118.19	77.76 a	44.51 a	16.65
F-value		ns	ns	*	**	ns

[a] WAT = weeks after first application.

[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.4. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of a perennial biotype of annual bluegrass under controlled conditions as influenced by various surfactants.

		Clipping fresh weight (% of untreated control)				
		WAT[a]				
Treatment	% (V/V)	0	1	2	3	4
Xanthomonas campestris[b] alone	1.0	115.74 a	62.26 b	61.83 b	38.23 b	19.17 ab
Agri-dex	0.5	75.81 a	74.69 ab	58.24 b	36.99 b	23.97 a
+						
Xanthomonas campestris	1.0					
Break-thru	0.75	88.94 a	91.09 ab	33.71 b	8.45 c	0.00 b
+						
Xanthomonas campestris	1.0					
Silwet L-77	0.25	130.01 a	108.34 a	57.39 b	52.90 ab	33.78 a
+						
Xanthomonas campestris	1.0					
X-77	0.5	97.24 a	93.33 ab	98.18 a	65.04 a	33.73 a
+						
Xanthomonas campestris	1.0					
F-value		ns	ns	*	**	*

[a] WAT = weeks after first application.

[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

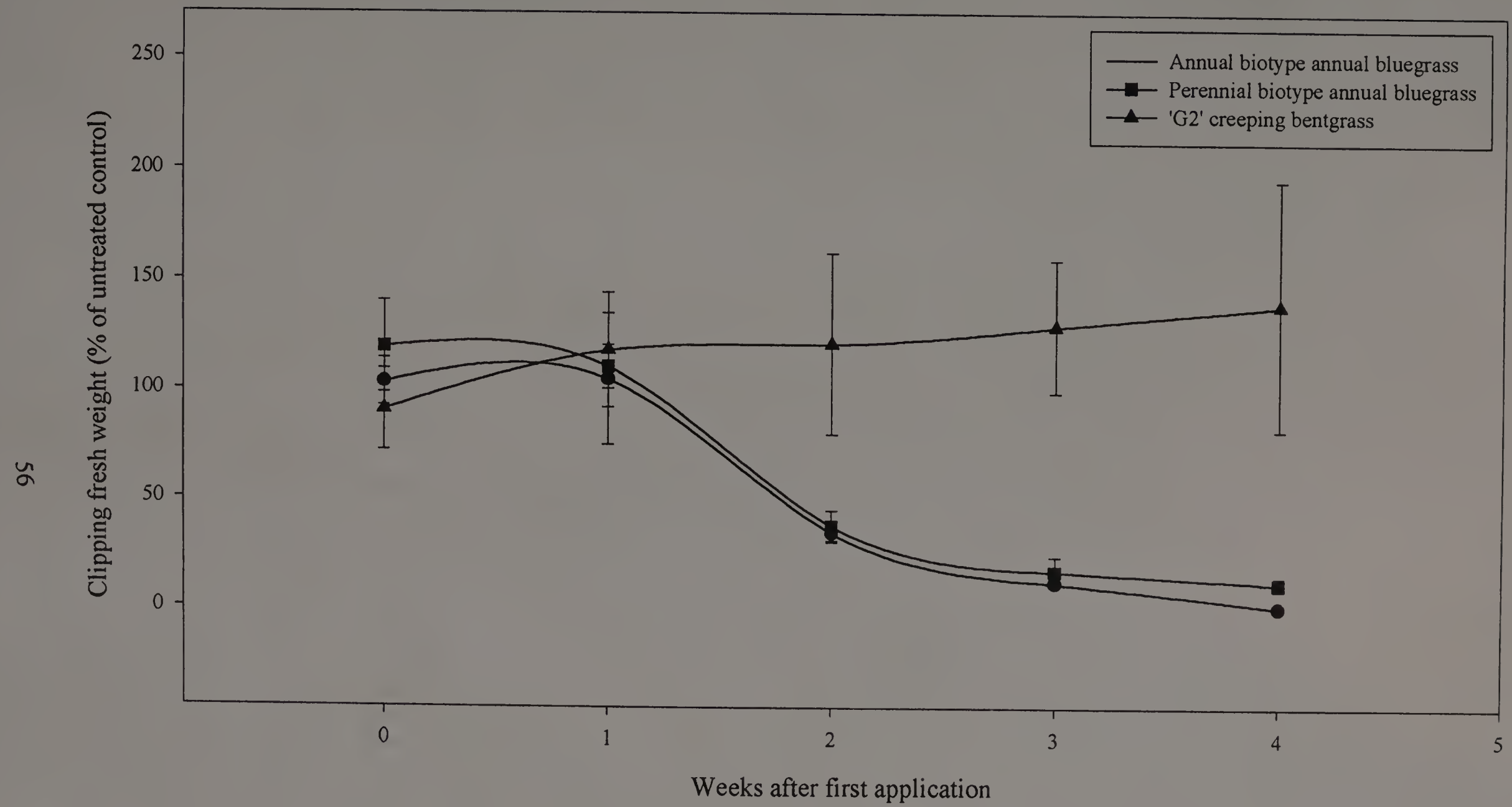

Figure 3.2. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weights of creeping bentgrass and two biotypes of annual bluegrass under controlled conditions.

Table 3.5. Effects of *Xanthomonas campestris* pv. *poannua* on quality of 'G2' creeping bentgrass under controlled conditions as influenced by various surfactants.

Treatment	% (V/V)	Turfgrass quality ratings[a] WAT[b] 0	1	2	3	4
Untreated control		9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
Xanthomonas campestris[c] alone	1.0	9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
Agri-dex	0.5	9.00 a	8.75 a	9.00 a	9.00 a	9.00 a
+						
Xanthomonas campestris	1.0					
X. c. p. + Break-thru	0.75	6.00 d	7.25 b	5.25 b	2.00 b	2.00 b
+						
Xanthomonas campestris	1.0					
X. c. p. + Silwet L-77	0.25	7.25 b	8.50 a	8.75 a	9.00 a	9.00 a
+						
Xanthomonas campestris	1.0					
X. c. p. + X-77	0.5	6.50 c	8.25 a	9.00 a	9.00 a	9.00 a
+						
Xanthomonas campestris	1.0					
F-value		**	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.6. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of 'G2' creeping bentgrass under controlled conditions as influenced by various surfactants.

Treatment	% (V/V)	Clipping fresh weight (% of untreated control) WAT[a] 0	1	2	3	4
Xanthomonas campestris[b] alone	1.0	132.50 a	152.17 a	87.48 a	126.96 a	130.37 a
Agri-dex + *Xanthomonas campestris*	0.5 + 1.0	118.82 a	124.17 a	110.46 a	152.74 a	164.03 a
Break-thru + *Xanthomonas campestris*	0.75 + 1.0	129.39 a	155.96 a	53.08 b	25.73 b	18.26 b
Silwet L-77 + *Xanthomonas campestris*	0.25 + 1.0	116.55 a	119.52 a	97.71 a	155.33 a	147.36 a
X-77 + *Xanthomonas campestris*	0.5 + 1.0	121.51 a	151.64 a	119.02 a	138.13 a	161.36 a
F-value		ns	ns	**	**	**

[a] WAT = weeks after first application.

[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.7. Effects of *Xanthomonas campestris* pv. *poannua* on seedhead production by a perennial biotype of annual bluegrass under controlled conditions as influenced by various surfactants.

		Percent seedhead supression[a]				
		WAT[b]				
Treatment	% (V/V)	0	1	2	3	4
Untreated control		20.00	-12.50	22.50	3.75	1.25
Xanthomonas campestris[c] alone	1.0	6.25	1.25	25.00	0.00	0.00
Agri-dex	0.5	0.00	2.50	6.25	5.00	11.25
+						
Xanthomonas campestris	1.0					
Break-thru	0.75	15.00	22.50	1.25	0.00	6.00
+						
Xanthomonas campestris	1.0					
Silwet L-77	0.25	10.00	-5.00	5.00	1.25	0.00
+						
Xanthomonas campestris	1.0					
X-77	0.5	-10.00	6.25	8.75	5.00	1.25
+						
Xanthomonas campestris	1.0					
F-value		ns	ns	ns	ns	ns

[a] Refers to the reduction in percentage of shoots with seedheads between the previous rating and the current rating.
[b] WAT = to weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Experiment No. 1.

Tank mixing of Agri-dex and the bacterial suspension at 0.5 % (V/V) reduced the quality and clipping fresh weight of the annual biotype of annual bluegrass by 40% (Table 3.8) and 50% (Table 3.3) 2 WAT, respectively, but these effects subsided by 3 WAT. Otherwise, the effects of treatments receiving Agri-dex in combination with *Xanthomonas campestris* were not significantly different (P=0.05) from those receiving the bacterium alone throughout the course of the experiment.

The addition of Break-thru at 0.75% (V/V) had little or no effect on suppression, clipping fresh weight, or quality of the annual biotype of annual bluegrass (Tables 3.1, 3.3, and 3.8; Figures 3.1 and 3.3). Clipping fresh weight and quality of the perennial biotype of annual bluegrass were reduced by 19 and 29% 4 WAT, respectively, as compared to the bacterium alone. However, suppression of the perennial biotype was not affected (Tables 3.4, 3.9, and 3.2; Figures 3.1, 3.4 and 3.5). The combination of *Xanthomonas campestris* and Break-thru at 0.75% (V/V) injured creeping bentgrass, while clipping fresh weight and quality of creeping bentgrass were reduced by 88 and 80% 3 WAT, respectively (Tables 3.5 and 3.6; Figure 3.5).

The bacterial suspension combined with Silwet L-77 at 0.25% (V/V), increased clipping fresh weight of the annual biotype of annual bluegrass by 15% 3 WAT, as compared to the bacterium alone (Table 3.3). Otherwise, Silwet L-77 did not influence the activity of *Xanthomonas campestris* in suppression of annual bluegrass or the quality of creeping bentgrass (Tables 3.1 to 3.9). X-77 in combination with the bacterial

Table 3.8. Effects of *Xanthomonas campestris* pv. *poannua* on quality of an annual biotype of annual bluegrass under controlled conditions as influenced by various surfactants.

Treatment	% (V/V)	Turfgrass quality ratings[a] WAT[b] 0	1	2	3	4
Untreated Control		9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
Xanthomonas campestris[c] alone	1.0	9.00 a	9.00 a	7.75 ab	2.00 b	2.00 b
Agridex	0.5	9.00 a	8.50 a	4.50 e	2.00 b	2.00 b
+						
Xanthomonas campestris	1.0					
Break-thru	0.75	6.50 b	7.00 c	5.00 de	2.00 b	1.50 b
+						
Xanthomonas campestris	1.0					
Silwet L-77	0.25	6.75 b	7.75 b	6.75 bc	2.25 b	2.00 b
+						
Xanthomonas campestris	1.0					
X-77	0.5	6.50 b	7.25 bc	6.25 cd	2.25 b	1.75 b
+						
Xanthomonas campestris	1.0					
F-value		**	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

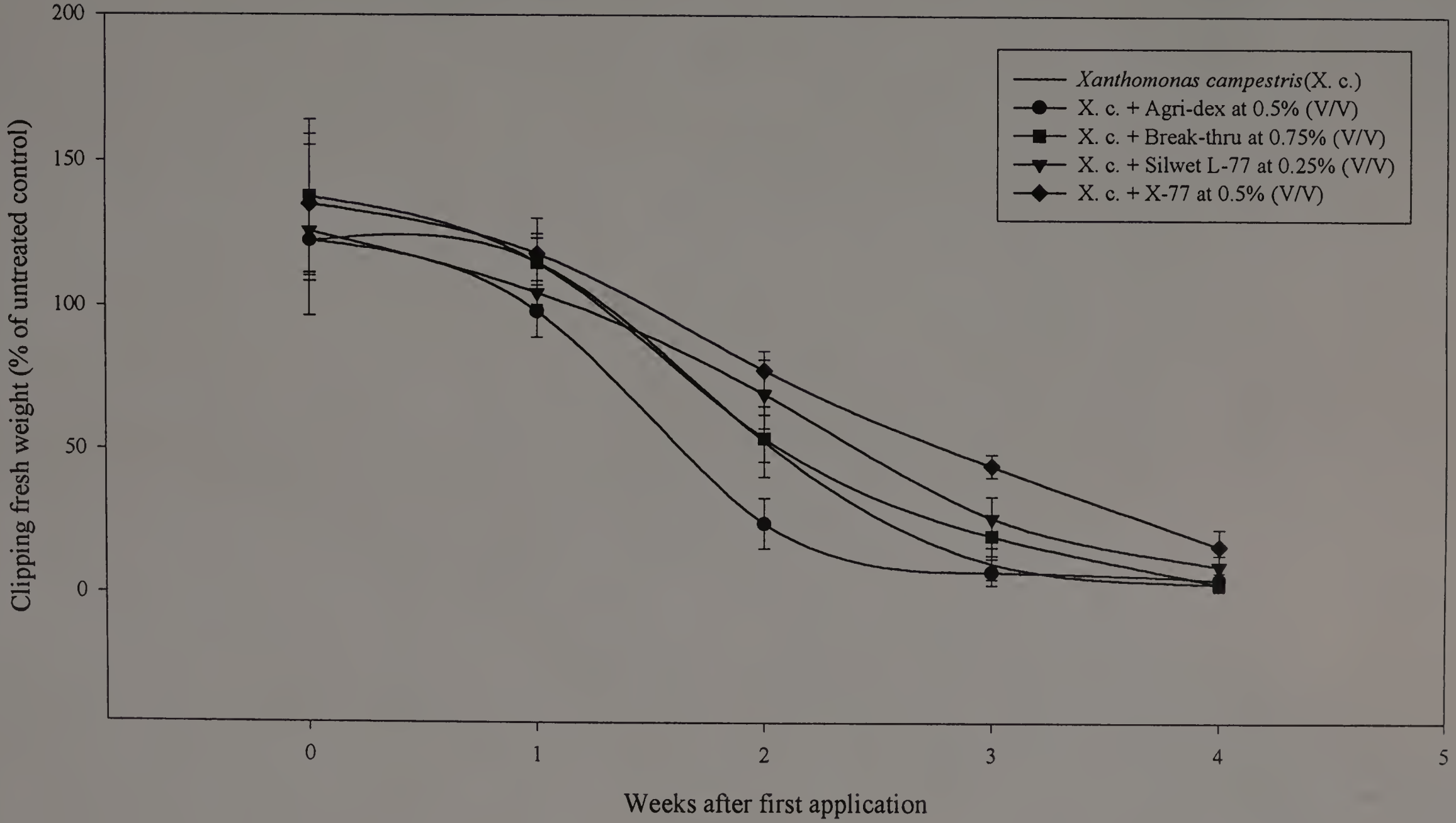

Figure 3.3. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of an annual biotype of annual bluegrass under controlled conditions as influenced by various surfactants.

Table 3.9. Effects of *Xanthomonas campestris* pv. *poannua* on quality of a perennial biotype of annual bluegrass under controlled conditions as influenced by various surfactants.

		Turfgrass quality ratings[a]				
		WAT[b]				
Treatment	% (V/V)	0	1	2	3	4
Untreated Control		9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
Xanthomonas campestris[c] alone	1.0	9.00 a	8.75	8.25 ab	5.00 bc	3.25 b
Agridex	0.5	9.00 a	8.75	7.00 b	4.25 cd	4.00 b
+						
Xanthomonas campestris	1.0					
Break-thru	0.75	7.25 b	7.75	5.25 c	2.50 d	1.00 c
+						
Xanthomonas campestris	1.0					
Silwet L-77	0.25	7.25 b	8.50	8.50 ab	6.75 b	5.25 b
+						
Xanthomonas campestris	1.0					
X-77	0.5	7.50 b	8.25	8.25 ab	6.50 b	4.50 b
+						
Xanthomonas campestris	1.0					
F-value		**	ns	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

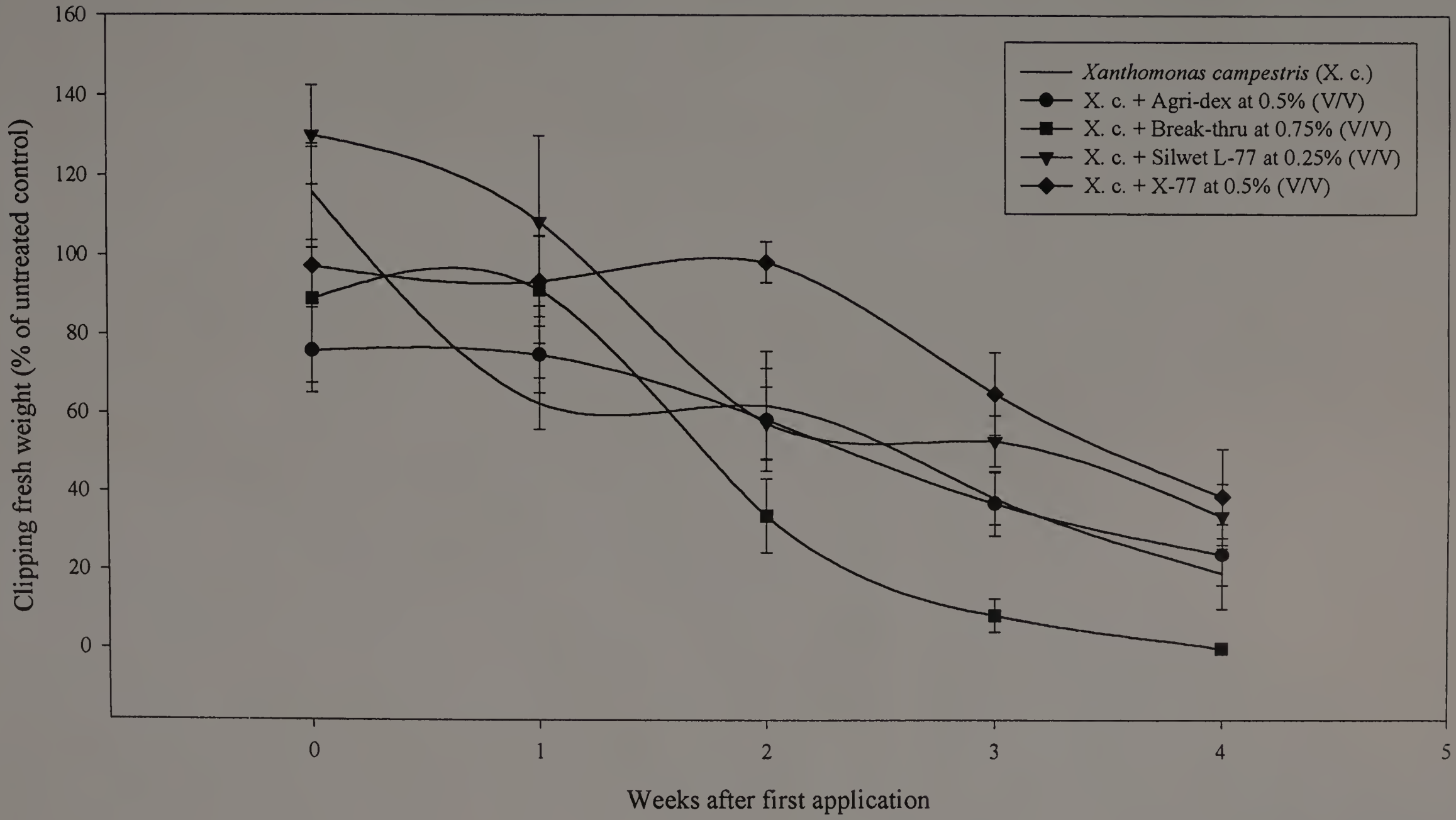

Figure 3.4. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of a perennial biotype of annual bluegrass under controlled conditions as influenced by various surfactants.

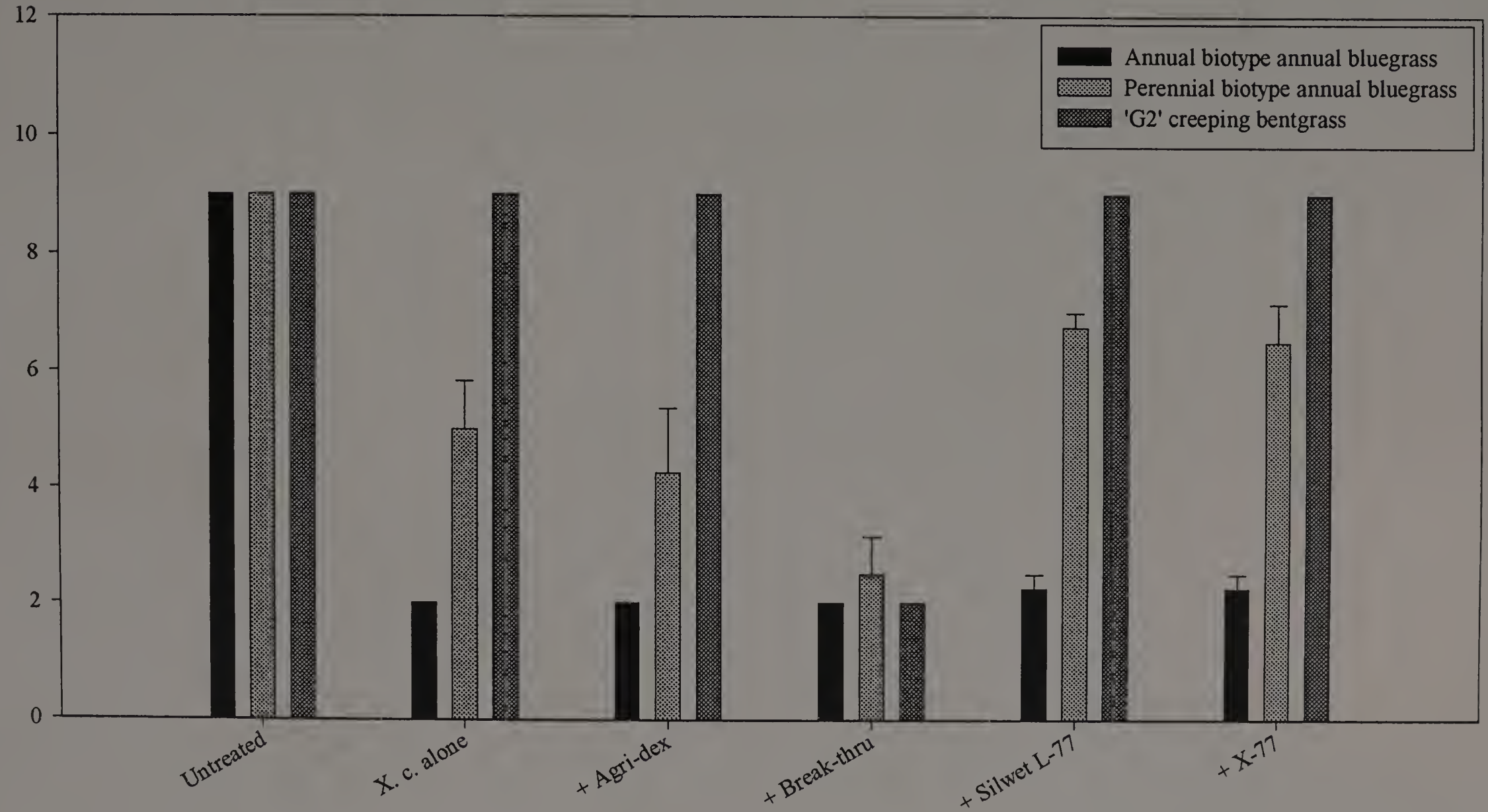

Figure 3.5. Effects of *Xanthomonas campestris* pv. *poannua* on quality of creeping bentgrass and two biotypes of annual bluegrass under contolled conditions as influenced by various surfactants 3 WAT.

by 57% 7 WAT (Table 3.2 and Figure 3.1). X-77 did not affect the quality of annual bluegrass or creeping bentgrass, or the clipping fresh weight of creeping bentgrass (Tables 3.5, 3.6, 3.8, and 3.9).

Experiment No. 2.

Break-thru at 0.3% (V/V) in combination with the bacterial suspension had no effect on suppression of annual bluegrass or the quality of creeping bentgrass, compared to treatments receiving only *Xanthomonas campestris* (Tables 3.10 to 3.18; Figures 3.5 to 3.9). Break-thru at higher rates [0.6 and 0.9% (V/V)] inhibited the activity of *Xanthomonas campestris*, and injured creeping bentgrass (Tables 3.10 to 3.18; Figures 3.5 to 3.9). Treatments of Break-thru at 0.6 and 0.9% (V/V) suppressed of the annual biotype of annual bluegrass by 83 and 61% 7 WAT, respectively, and the perennial biotype by 70 and 12% 7 WAT, respectively (Tables 3.10 and 3.11; Figure 3.6). Bentgrass quality was also reduced by 21 and 31% 3 WAT, respectively, by the treatment combinations of *Xanthomonas campestris* and Break-thru at 0.6 and 0.9% (V/V) (Table 3.18 and Figure 3.9).

Neither Rely nor Rewet influenced the activity of *Xanthomonas campestris* in the suppression of annual bluegrass, or on the quality or clipping fresh weight of creeping bentgrass (Tables 3.10 to 3.18).

Table 3.10. Suppression of an annual biotype of annual bluegrass by *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Percent weed suppression WAT[a] 3	4	7
Untreated control		0.00 e	0.00 d	0.00 d
Xanthomonas campestris[b]	9.46	91.25 a	99.25 a	100.00 a
Rely + *Xanthomonas campestris*	6.44 9.46	73.75 ab	97.00 a	100.00 a
Rely + *Xanthomonas campestris*	12.88 9.46	86.25 a	96.00 a	93.75 ab
Rely + *Xanthomonas campestris*	19.32 9.46	84.75 a	96.75 a	97.00 ab
Rewet + *Xanthomonas campestris*	16.10 9.46	91.00 a	98.25 a	99.75 a
Rewet + *Xanthomonas campestris*	22.54 9.46	90.00 a	97.00 a	98.50 a
Rewet + *Xanthomonas campestris*	32.20 9.46	89.75 a	96.75 a	98.50 a
Break-thru[c] + *Xanthomonas campestris*	0.3% 9.46	55.00 bc	97.00 a	99.50 a
Break-thru + *Xanthomonas campestris*	0.6% 9.46	33.75 cd	81.25 b	83.75 b
Break-thru + *Xanthomonas campestris*	0.9% 9.46	6.25 de	65.00 c	61.25 c
F-value		**	**	**

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[c] Applied V/V.

Table 3.11. Suppression of a perennial biotype of annual bluegrass by *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Percent weed suppression WAT[a] 3	4	7
Untreated control		0.00 c	0.00 d	0.00 c
Xanthomonas campestris[b]	9.46	76.25 a	100.00 a	100.00 a
Rely + *Xanthomonas campestris*	6.44 9.46	71.00 ab	99.75 a	100.00 a
Rely + *Xanthomonas campestris*	12.88 9.46	82.50 a	100.00 a	98.75 a
Rely + *Xanthomonas campestris*	19.32 9.46	38.75 b	93.75 ab	98.75 a
Rewet + *Xanthomonas campestris*	16.10 9.46	63.75 ab	100.00 a	98.75 a
Rewet + *Xanthomonas campestris*	22.54 9.46	53.75 ab	100.00 a	100.00 a
Rewet + *Xanthomonas campestris*	32.20 9.46	57.25 ab	100.00 a	100.00 a
Break-thru[c] + *Xanthomonas campestris*	0.3% 9.46	7.50 c	87.50 b	93.75 a
Break-thru + *Xanthomonas campestris*	0.6% 9.46	5.00 c	16.25 c	70.00 b
Break-thru + *Xanthomonas campestris*	0.9% 9.46	0.00 c	5.00 d	12.50 c
F-value		**	**	**

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[c] Applied V/V.

Table 3.12. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of an annual biotype of annual bluegrass under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Clipping fresh weight (% of untreated control), WAT[a]				
		0	1	2	3	4
Xanthomonas campestris[b] alone	9.46	103.29 ab	104.71 ab	34.28 cd	11.69 b	0.58 c
Rely	6.44	107.16 ab	79.45 b	34.59 cd	26.04 b	2.78 c
+ *Xanthomonas campestris*	9.46					
Rely	12.88	100.81 ab	112.72 ab	32.71 cd	21.63 b	4.10 c
+ *Xanthomonas campestris*	9.46					
Rely	19.32	111.57 ab	89.03 b	54.29 cd	24.04 b	3.36 c
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	74.67 c	103.30 ab	29.61 d	11.46 b	1.75 c
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	91.09 bc	147.19 a	29.81 d	12.47 b	5.57 c
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	92.80 bc	136.11 a	23.94 d	23.07 b	0.00 c
+ *Xanthomonas campestris*	9.46					
Break-thru[c]	0.3%	99.63 ab	138.97 a	61.70 b	37.90 b	7.10 c
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	113.95 ab	141.34 a	94.86 a	84.85 a	35.81 b
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	117.29 a	118.20 ab	89.57 a	90.83 a	60.03 a
+ *Xanthomonas campestris*	9.46					
F-value		**	*	**	**	**

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[c] Applied V/V.

Table 3.13. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of a perennial biotype of annual bluegrass under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Clipping fresh weight (% of untreated control), WAT[a]				
		0	1	2	3	4
Xanthomonas campestris[b] alone	9.46	119.36 ab	110.35	37.42 c	17.01 b	11.45 c
Rely	6.44	142.32 a	104.18	46.93 bc	23.70 b	11.02 c
+ *Xanthomonas campestris*	9.46					
Rely	12.88	147.43 a	106.15	35.26 c	14.12 b	5.68 c
+ *Xanthomonas campestris*	9.46					
Rely	19.32	92.94 b	97.89	47.17 bc	33.74 b	7.22 c
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	86.22 b	98.20	35.53 c	23.05 b	4.07 c
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	80.19 b	119.58	45.28 c	38.14 b	10.37 c
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	80.00 b	91.81	38.41 c	19.83 b	7.66 c
+ *Xanthomonas campestris*	9.46					
Break-thru[c]	0.3%	148.30 a	102.01	92.07 a	83.63 a	18.57 c
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	116.46 ab	95.72	91.61 a	77.47 a	44.94 b
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	108.08 ab	87.63	71.95 ab	81.63 a	70.37 a
+ *Xanthomonas campestris*	9.46					
F-value		*	ns	**	**	**

[a] WAT = weeks after first application.

[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

[c] Applied V/V.

Table 3.14. Effects of *Xanthomonas campestris* pv. *poannua* on seedhead production by a perennial biotype of annual bluegrass under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Turfgrass quality rating[a], WAT[b]				
		0	1	2	3	4
Untreated control		-17.50	41.25 abc	11.25	6.25	-2.50
Xanthomonas campestris[c] alone	9.46	-11.00	18.25 abc	8.75	2.50	0.00
Rely	6.44	21.25	6.25 c	3.75	2.50	0.00
+ *Xanthomonas campestris*	9.46					
Rely	12.88	0.00	16.25 abc	2.50	1.25	0.00
+ *Xanthomonas campestris*	9.46					
Rely	19.32	-22.50	50.00 a	12.50	1.25	0.00
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	6.25	5.00 c	6.25	5.00	0.00
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	16.25	6.25 c	1.25	3.75	0.00
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	21.00	7.50 c	5.00	5.00	0.00
+ *Xanthomonas campestris*	9.46					
Break-thru[d]	0.3%	-2.75	10.25 bc	1.25	1.25	1.25
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	1.25	33.75 abc	3.75	2.50	0.00
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	-3.25	46.00 ab	1.25	5.00	-1.25
+ *Xanthomonas campestris*	9.46					
F-value		ns	*	ns	ns	ns

[a] Refers to the reduction in percentage of shoots with seedheads between the previous rating and the current rating.
[b] WAT = weeks after first application. [d] Applied V/V.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.15. Effects of *Xanthomonas campestris* pv. *poannua* on quality of an annual biotype of annual bluegrass under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Turfgrass quality rating[a], WAT[b]				
		0	1	2	3	4
Untreated control		9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
Xanthomonas campestris[c] alone	9.46	8.25 a	8.75 a	4.75 c	2.00 d	1.75 c
Rely	6.44	8.25 a	7.50 b	4.00 cd	2.50 d	1.75 c
+ *Xanthomonas campestris*	9.46					
Rely	12.88	7.25 b	6.75 bc	3.75 cd	2.00 d	1.75 c
+ *Xanthomonas campestris*	9.46					
Rely	19.32	6.75 b	6.25 c	4.50 cd	2.50 d	2.00 c
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	9.00 a	7.00 bc	3.25 c	2.50 d	1.75 c
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	9.00 a	7.00 bc	3.00 d	2.00 d	2.00 c
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	9.00 a	7.00 bc	3.00 d	2.00 d	2.00 c
+ *Xanthomonas campestris*	9.46					
Break-thru[d]	0.3%	7.25 b	7.50 b	6.00 b	3.00 cd	2.00 c
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	7.00 b	7.50 b	6.50 b	4.25 c	3.00 b
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	6.75 b	6.25 c	6.00 b	6.25 b	3.50 b
+ *Xanthomonas campestris*	9.46					
F-value		**	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[d] Applied V/V.

Table 3.16. Effects of *Xanthomonas campestris* pv. *poannua* on quality of a perennial biotype of annual bluegrass under controlled conditions as influenced by various surfactants and their rates

Treatment	Rate (L ha^{-1})	Turfgrass quality rating[a], WAT[b]				
		0	1	2	3	4
Untreated control		9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
Xanthomonas campestris[c] alone	9.46	9.00 a	8.75 a	6.25 c	3.00 c	1.00 d
Rely	6.44	8.75 a	8.00 ab	6.00 cd	3.50 c	1.00 d
+ *Xanthomonas campestris*	9.46					
Rely	12.88	7.75 ab	6.00 c	4.75 d	2.00 c	1.00 d
+ *Xanthomonas campestris*	9.46					
Rely	19.32	6.50 c	6.75 bc	5.57 cd	3.25 c	1.50 d
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	9.00 a	7.75 ab	6.50 bc	3.00 c	1.00 d
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	9.00 a	7.00 bc	5.75 cd	3.50 c	1.00 d
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	9.00 a	7.75 ab	5.75 cd	3.75 c	1.00 d
+ *Xanthomonas campestris*	9.46					
Break-thru[d]	0.3%	8.25 ab	8.00 ab	9.00 a	6.50 b	1.75 d
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	7.50 b	6.75 bc	7.75 ab	6.25 b	5.50 c
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	6.50 b	6.25 c	6.50 bc	6.75 b	7.50 b
+ *Xanthomonas campestris*	9.46					
F-value		**	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}, the spray volume was 946 L ha^{-1}.
[d] Applied V/V.

Table 3.17. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of 'G2' creeping bentgrass under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Clipping fresh weight (% of untreated control), WAT[a]				
		0	1	2	3	4
Xanthomonas campestris[b] alone	9.46	90.63	117.89	120.91	129.13	138.77
Rely	6.44	109.22	108.68	111.85	163.89	202.00
+ *Xanthomonas campestris*	9.46					
Rely	12.88	101.84	152.26	104.90	158.90	193.28
+ *Xanthomonas campestris*	9.46					
Rely	19.32	125.50	99.13	107.18	139.89	122.15
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	81.63	117.19	104.68	173.71	222.70
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	69.73	145.31	110.91	179.26	204.31
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	77.48	132.29	82.65	135.69	145.24
+ *Xanthomonas campestris*	9.46					
Break-thru[c]	0.3%	97.18	134.90	80.48	136.41	173.42
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	98.58	163.71	130.54	177.51	186.14
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	113.74	137.15	102.59	158.70	134.67
+ *Xanthomonas campestris*	9.46					
F-value		ns	ns	ns	ns	ns

[a] WAT = weeks after first application.

[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

[c] Applied V/V.

Table 3.18. Effects of *Xanthomonas campestris* pv. *poannua* on quality of 'G2' creeping bentgrass under controlled conditions as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Turfgrass quality rating[a], WAT[b]				
		0	1	2	3	4
Untreated control		9.00 a	9.00 a	9.00	9.00 a	9.00 a
Xanthomonas campestris[c] alone	9.46	8.75 a	9.00 a	8.00	9.00 a	8.75 a
Rely	6.44	8.75 a	7.75 bc	8.25	9.00 a	9.00 a
+ *Xanthomonas campestris*	9.46					
Rely	12.88	7.75 bc	7.50 bc	7.50	8.75 ab	9.00 a
+ *Xanthomonas campestris*	9.46					
Rely	19.32	7.50 c	6.50 de	8.25	8.25 ab	8.25 a
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	9.00 a	7.25 cd	8.50	9.00 a	9.00 a
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	9.00 a	7.50 cd	8.25	9.00 a	9.00 a
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	9.00 a	7.50 cd	7.25	8.00 bc	9.00 a
+ *Xanthomonas campestris*	9.46					
Break-thru[d]	0.3%	8.00 b	8.25 ab	9.00	8.00 bc	8.50 a
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	7.00 d	6.50 de	7.50	7.25 cd	8.00 a
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	6.00 e	6.00 e	8.25	6.50 d	6.75 b
+ *Xanthomonas campestris*	9.46					
F-value		**	**	ns	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[d] Applied V/V.

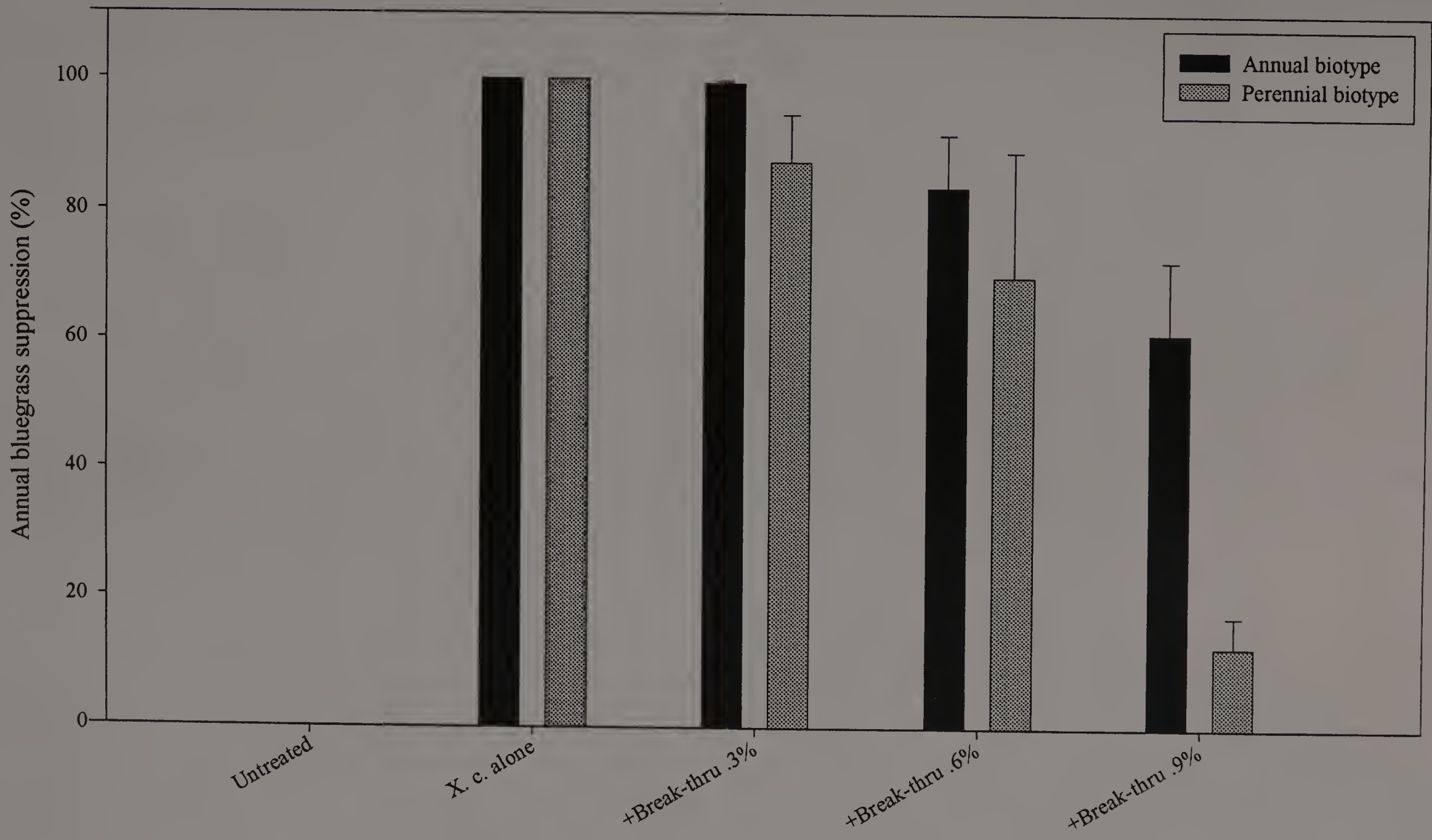

Figure 3.6. Activity of *Xanthomonas campestris* pv. *poannua* in suppression of two biotypes of annual bluegrass under controlled conditions as influenced by three rates of Break-thru 7 WAT.

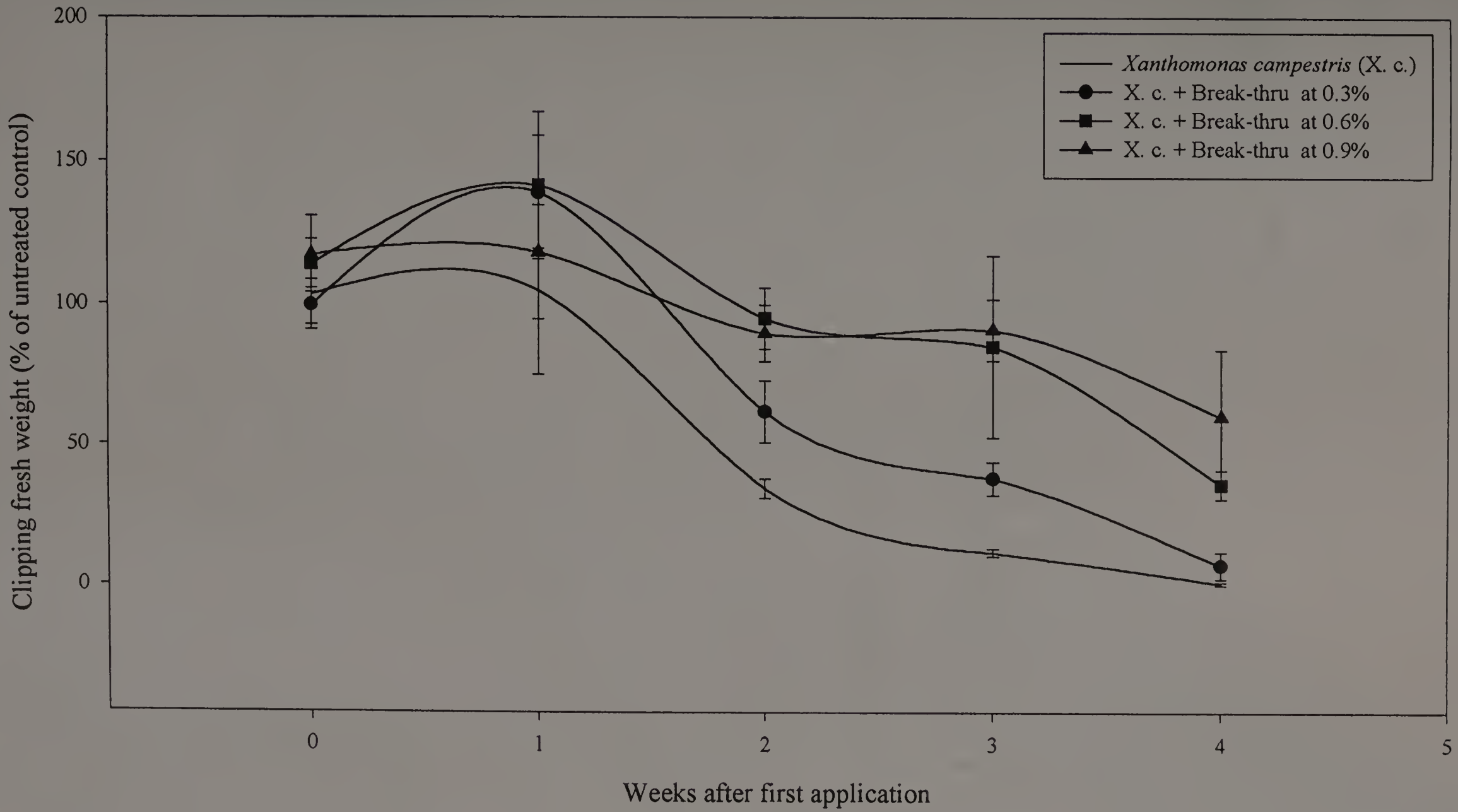

Figure 3.7. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of an annual biotype of annual bluegrass under controlled conditions as influenced by three rates of Break-thru.

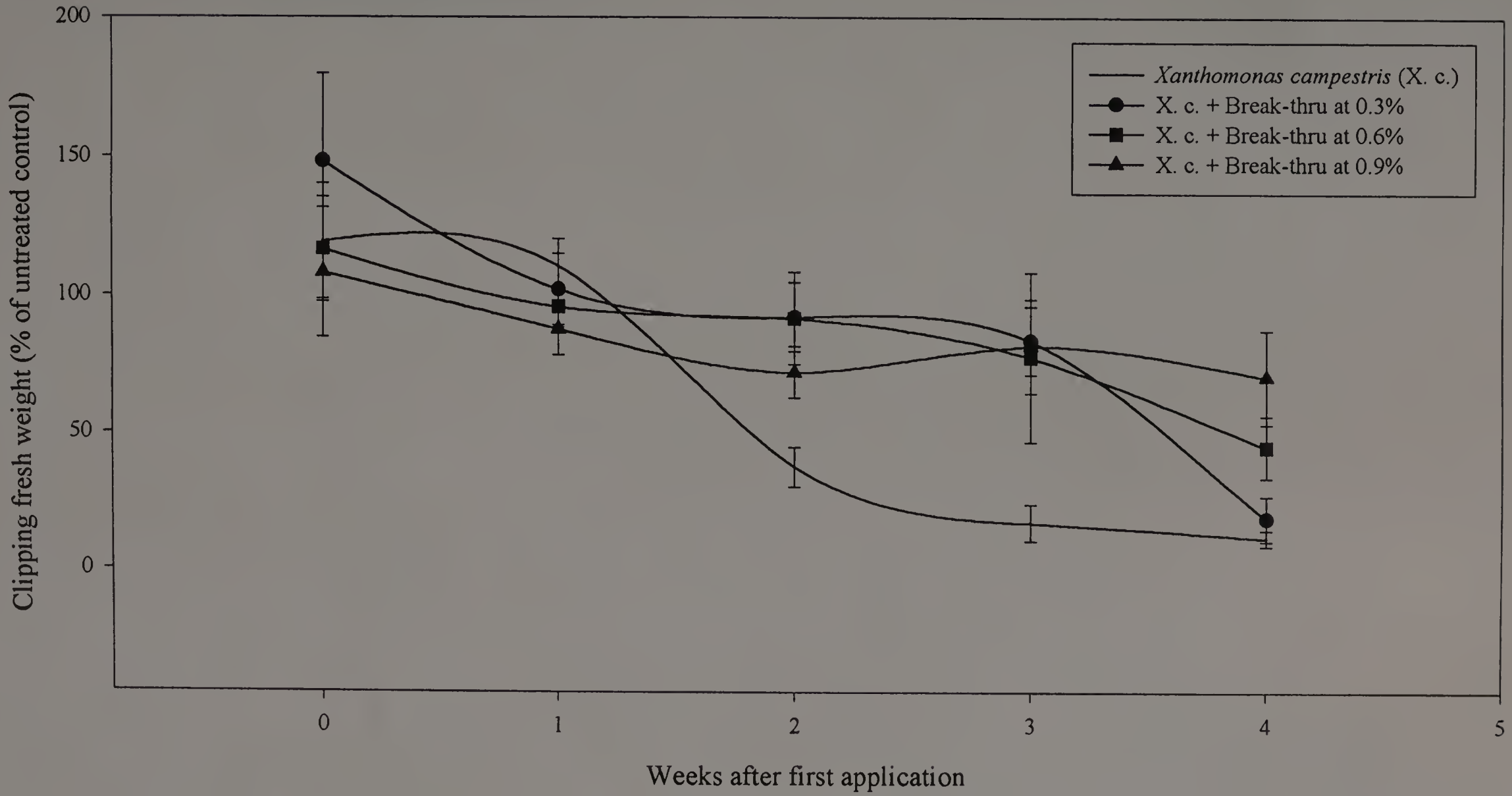

Figure 3.8. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weight of a perennial biotype of annual bluegrass under controlled conditions as influenced by three rates of Break-thru.

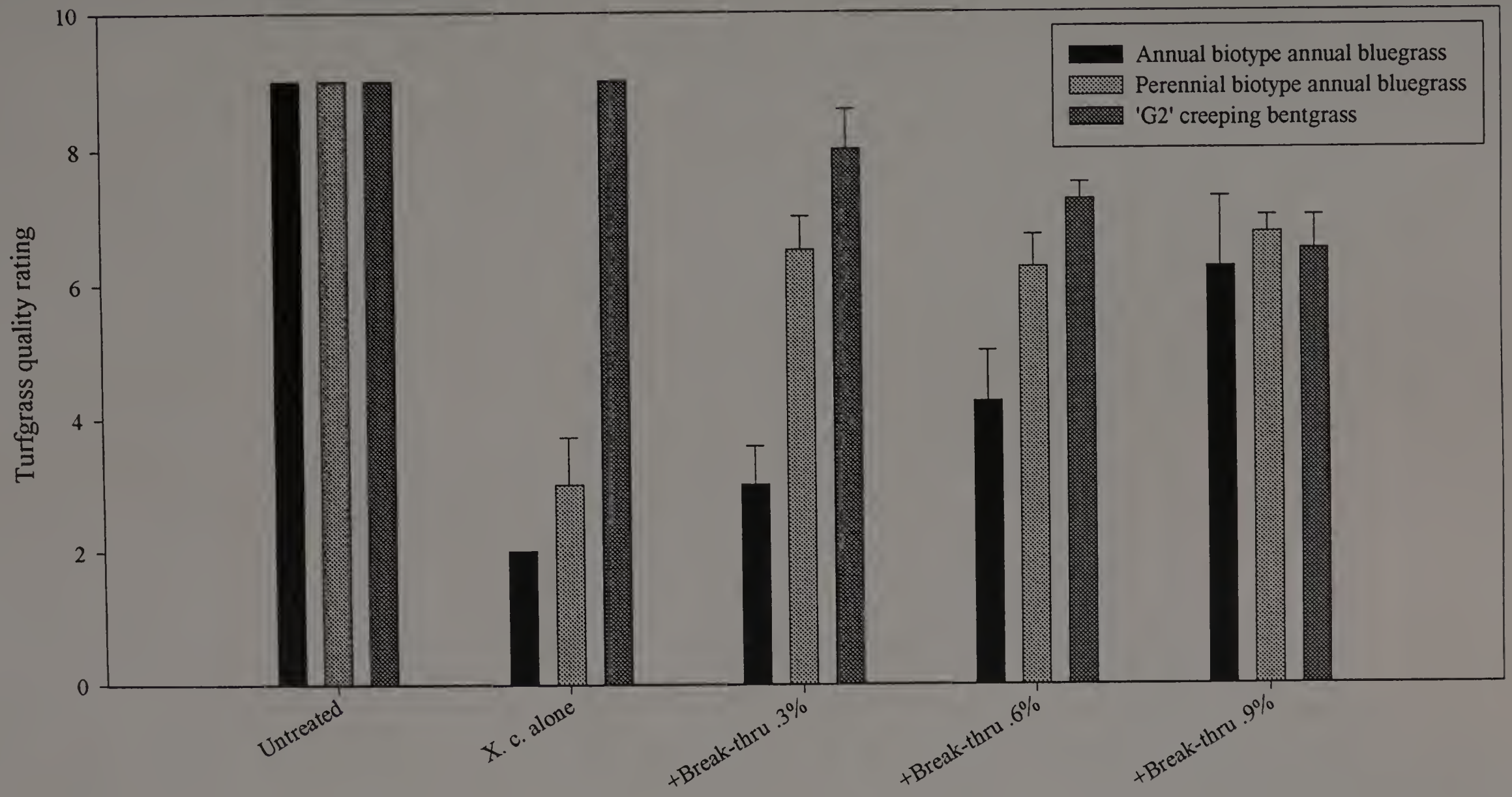

Figure 3.9. Effects of *Xanthomonas campestris* pv. *poannua* on quality of creeping bentgrass and two biotypes of annual bluegrass under controlled conditions as influenced by three rates of Break-thru 3 WAT.

Field experiments

Xanthomonas campestris applied at five weekly intervals, and at 10^7 cfu ml^{-1} did not suppress annual bluegrass (Tables 3.19 and 3.23) or reduce the quality of annual bluegrass (Tables 3.20 and 3.24; Figure 3.10) compared to the untreated control. The bacterium reduced seedhead production by 19% 7 WAT, but in no other instance (Tables 3.21 and 3.25). The quality of creeping bentgrass was not affected by the bacterium alone at any point during the field trials (Tables 3.22 and 3.26).

Experiment No. 1.

Agri-dex at 0.5% (V/V) did not affect growth (Table 3.19), quality (Table 3.20), or seedhead production of annual bluegrass (Table 3.21), or the quality of creeping bentgrass (Table 3.22). Silwet L-77, in combination with *Xanthomonas campestris*, reduced the quality of annual bluegrass and creeping bentgrass by 12 and 25% 5 and 4 WAT, respectively (Table 3.20), but had no effect on annual bluegrass suppression (Table 3.19). Similarly, X-77 had no harmful effects on annual bluegrass (Table 3.19), but suppressed seedhead production by 30% 7 WAT (Table 3.21). Treatments of *Xanthomonas campestris* in combination with X-77 at 0.5% (V/V) resulted in reduced annual bluegrass and creeping bentgrass quality of 12% 4 WAT (Table 3.20) and 20% 7 WAT (Table 3.22), respectively.

Table 3.19. Effects of *Xanthomonas campestris* pv. *poannua* on annual bluegrass encroachment on a 'Providence' creeping bentgrass putting green as influenced by various surfactants.

		Percent annual bluegrass increase[a], WAT[b]				
Treatment	Rate [% (V/V)]	3	4	5	7	9
Untreated Control		18.33 a	12.17 a	3.00 a	16.33 a	16.33 a
Xanthomonas campestris[c] alone	1.0	16.33 ab	11.33 a	13.00 a	13.00 ab	13.83 ab
Agridex	0.5	13.83 ab	10.50 a	11.33 ab	13.83 ab	13.83 ab
+						
Xanthomonas campestris	1.0					
Break-thru	0.75	6.33 c	2.17 b	4.67 c	6.33 c	2.17 c
+						
Xanthomonas campestris	1.0					
Silwet L-77	0.25	9.67 bc	8.13 a	9.67 abc	10.50 b	8.83 b
+						
Xanthomonas campestris	1.0					
X-77	0.5	9.67 bc	7.17 b	6.33 bc	10.50 b	13.83 ab
+						
Xanthomonas campestris	1.0					
F-value		**	**	**	**	**

[a] Cumulative increase in plot area covered by annual bluegrass compared to 0 WAT.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.20. Effects of *Xanthomonas campestris* pv. *poannua* on the quality of annual bluegrass on a 'Providence' creeping bentgrass putting green as influenced by various surfactants.

Treatment	Rate [% (V/V)]	Turfgrass quality rating[a], WAT[b] 3	4	5	7	9
Untreated Control		9.00	9.00 a	9.00 a	9.00 a	9.00 a
Xanthomonas campestris[c] alone	1.0	9.00	9.00 a	9.00 a	8.83 a	9.00 a
Agridex	0.5	9.00	9.00 a	8.83 a	9.00 a	9.00 a
+						
Xanthomonas campestris	1.0					
Break-thru	0.75	9.00	7.00 b	6.33 c	4.67 c	5.17 c
+						
Xanthomonas campestris	1.0					
Silwet L-77	0.25	9.00	9.00 a	8.00 b	6.33 b	6.00 b
+						
Xanthomonas campestris	1.0					
X-77	0.5	9.00	9.00 a	8.83 ab	7.33 b	9.00 a
+						
Xanthomonas campestris	1.0					
F-value		ns	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.21. Effects of *Xanthomonas campestris* pv. *poannua* on annual bluegrass seedhead production on a 'Providence' creeping bentgrass putting green as influenced by various surfactants.

Treatment	Rate [% (V/V)]	Percent seedhead production[a], WAT[b] 3	5	7
Untreated Control		53.33 ab	60.00 ab	76.67 a
Xanthomonas campestris[c] alone	1.0	55.83 ab	66.67 a	58.33 b
Agridex	0.5	62.50 a	50.83 ab	59.17 b
+				
Xanthomonas campestris	1.0			
Break-thru	0.75	41.67 b	20.83 c	32.50 c
+				
Xanthomonas campestris	1.0			
Silwet L-77	0.25	55.83 ab	49.17 ab	38.33 c
+				
Xanthomonas campestris	1.0			
X-77	0.5	64.17 a	44.17 b	44.17 bc
+				
Xanthomonas campestris	1.0			
F-value		*	**	**

[a] Refers to the percentage of annual bluegrass shoots bearing seedheads.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.22. Effects of *Xanthomonas campestris* pv. *poannua* on the quality of 'Providence' creeping bentgrass on a putting green as influenced by various surfactants.

Treatment	Rate [% (V/V)]	Turfgrass quality rating[a], WAT[b] 3	4	5	7	9
Untreated Control		9.00 a	9.00 a	9.00 a	9.00	9.00
Xanthomonas campestris[c] alone	1.0	9.00 a	9.00 a	9.00 a	9.00	9.00
Agridex	0.5	9.00 a	9.00 a	9.00 a	9.00	9.00
+						
Xanthomonas campestris	1.0					
Break-thru	0.75	6.00 b	4.00 d	7.17 b	9.00	9.00
+						
Xanthomonas campestris	1.0					
Silwet L-77	0.25	9.00 a	6.00 c	8.50 a	9.00	9.00
+						
Xanthomonas campestris	1.0					
X-77	0.5	9.00 a	7.00 b	8.67 a	9.00	9.00
+						
Xanthomonas campestris	1.0					
F-value		**	**	**	ns	ns

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.

Table 3.23. Effects of *Xanthomonas campestris* pv. *poannua* on annual bluegrass encroachment on a 'Penncross' creeping bentgrass putting green as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Percent annual bluegrass increase[a], WAT[b]				
		3	4	5	7	9
Untreated control		14.17 a	4.17	5.83 abc	11.67 a	11.67 a
Xanthomonas campestris[c] alone	9.46	10.00 ab	3.33	6.67 abc	10.00 ab	10.00 abc
Rely	6.44	5.00 cd	0.83	1.67 c	7.50 abc	9.17 abcd
+ *Xanthomonas campestris*	9.46					
Rely	12.88	6.67 bc	3.33	5.83 abc	8.33 abc	10.83 ab
+ *Xanthomonas campestris*	9.46					
Rely	19.32	4.17 cd	2.50	4.17 bc	6.67 abcd	8.33 abcd
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	0.83 d	-1.67	4.17 bc	3.33 cd	5.83 cdef
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	0.83 d	0.83	3.33 bc	3.33 cd	5.83 cdef
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	0.00 d	0.00	5.00 bc	5.00 bcd	5.00 def
+ *Xanthomonas campestris*	9.46					
Break-thru[d]	0.3%	0.83 d	1.67	8.33 ab	8.33 abc	6.67 bcde
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	0.00 d	0.00	7.50 ab	4.17 cd	3.33 ef
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	0.00 d	0.83	10.83 a	1.67 c	1.67 f
+ *Xanthomonas campestris*	9.46					
F-value		**	ns	*	**	**

[a] Cumulative increase in plot area covered by annual bluegrass compared to 0 WAT. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[d] Applied V/V.

Table 3.24. Effects of *Xanthomonas campestris* pv. *poannua* on the quality of annual bluegrass on a 'Penncross' creeping bentgrass putting green as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Turfgrass quality rating[a], WAT[b]				
		3	4	5	7	9
Untreated control		9.00	9.00 a	9.00 a	9.00 a	9.00 a
Xanthomonas campestris[c] alone	9.46	9.00	9.00 a	9.00 a	8.83 ab	9.00 a
Rely	6.44	9.00	9.00 a	9.00 a	8.83 ab	8.33 b
+ *Xanthomonas campestris*	9.46					
Rely	12.88	9.00	9.00 a	9.00 a	8.33 abc	7.67 c
+ *Xanthomonas campestris*	9.46					
Rely	19.32	9.00	9.00 a	9.00 a	7.67 cd	8.00 bc
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	9.00	9.00 a	9.00 a	8.17 abcd	8.50 ab
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	9.00	9.00 a	8.83 a	8.50 abc	8.50 ab
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	9.00	9.00 a	9.00 a	8.83 ab	8.50 ab
+ *Xanthomonas campestris*	9.46					
Break-thru[d]	0.3%	9.00	9.00 a	9.00 a	8.33 abc	8.50 ab
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	9.00	8.33 a	8.50 b	8.00 bcd	8.50 ab
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	9.00	6.83 b	6.00 c	7.33 d	8.00 bc
+ *Xanthomonas campestris*	9.46					
F-value		ns	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[d] Applied V/V.

Table 3.25. Effects of *Xanthomonas campestris* pv. *poannua* on annual bluegrass seedhead production on a 'Penncross' creeping bentgrass putting green as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Percent seedhead production[a], WAT[b]		
		3	5	7
Untreated control		63.33 a	85.00 a	87.50 a
Xanthomonas campestris[c] alone	9.46	55.00 ab	83.33 a	84.17 ab
Rely	6.44	50.83 abc	78.33 a	80.00 ab
+ *Xanthomonas campestris*	9.46			
Rely	12.88	31.67 e	79.17 a	81.67 ab
+ *Xanthomonas campestris*	9.46			
Rely	19.32	40.00 cde	82.50 a	80.83 ab
+ *Xanthomonas campestris*	9.46			
Rewet	16.10	35.83 de	85.00 a	79.17 ab
+ *Xanthomonas campestris*	9.46			
Rewet	22.54	46.67 bcd	78.33 a	75.00 bc
+ *Xanthomonas campestris*	9.46			
Rewet	32.20	32.50 de	70.83 ab	75.00 bc
+ *Xanthomonas campestris*	9.46			
Break-thru[d]	0.3%	27.50 e	59.17 b	78.33 abc
+ *Xanthomonas campestris*	9.46			
Break-thru	0.6%	12.50 f	41.67 c	68.33 cd
+ *Xanthomonas campestris*	9.46			
Break-thru	0.9%	11.67 f	15.00 d	64.17 d
+ *Xanthomonas campestris*	9.46			
F-value		**	**	**

[a] Refers to the percentage of annual bluegrass shoots bearing seedheads. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[d] Applied V/V.

Table 3.26. Effects of *Xanthomonas campestris* pv. *poannua* on the quality of 'Penncross' creeping bentgrass on a putting green as influenced by various surfactants and their rates.

Treatment	Rate (L ha^{-1})	Turfgrass quality rating[a], WAT[b]				
		3	4	5	7	9
Untreated control		9.00 a	9.00 a	9.00 a	9.00	9.00
Xanthomonas campestris[c] alone	9.46	9.00 a	9.00 a	9.00 a	9.00	9.00
Rely	6.44	9.00 a	9.00 a	9.00 a	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Rely	12.88	9.00 a	9.00 a	9.00 a	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Rely	19.32	9.00 a	9.00 a	9.00 a	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Rewet	16.10	9.00 a	9.00 a	9.00 a	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Rewet	22.54	9.00 a	9.00 a	8.67 ab	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Rewet	32.20	9.00 a	9.00 a	9.00 a	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Break-thru[d]	0.3%	9.00 a	9.00 a	9.00 a	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Break-thru	0.6%	7.00 b	8.33 b	8.33 b	9.00	9.00
+ *Xanthomonas campestris*	9.46					
Break-thru	0.9%	6.00 c	4.00 c	6.00 c	9.00	9.00
+ *Xanthomonas campestris*	9.46					
F-value		**	**	**	ns	ns

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy. [b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L ha^{-1}; the spray volume was 946 L ha^{-1}.
[d] Applied V/V.

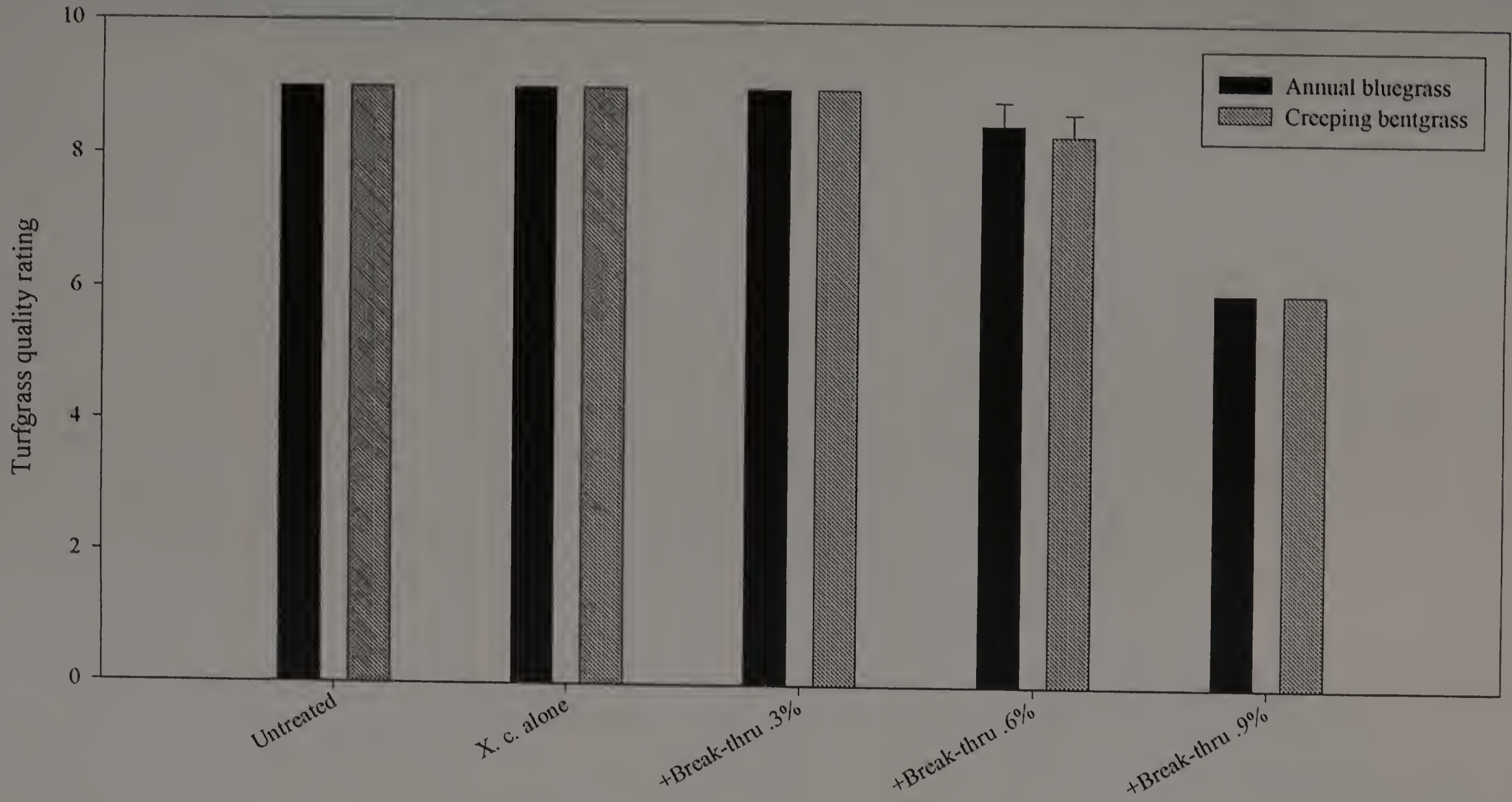

Figure 3.10. Effects of *Xanthomonas campestris* pv. *poannua* on quality of creeping bentgrass and annual bluegrass in a putting green as influenced by three rates of Break-thru 5 WAT.

Experiment No. 2.

The bacterial suspension in combination with Break-thru at 0.6 and 0.9% (V/V) suppressed annual bluegrass by 7 and 10% 9 WAT, respectively, compared to the untreated control (Table 3.23). At 5 WAT, Break-thru at 0.6 and 0.9% (V/V) reduced the quality of annual bluegrass by 6 and 25% (Table 3.24), reduced seedhead production by 30% at the highest rate (Table 3.25), and reduced the quality of creeping bentgrass by 8% and 25%, respectively (Table 3.26). Break-thru at the highest rate [0.9% (V/V)] injured (50% reduction in quality 4 WAT) creeping bentgrass (Table 3.26).

Treatment combinations of *Xanthomonas campestris* and Rely at 12.88 and 19.32 L ha^{-1} suppressed seedhead production by 31 and 23% 3 WAT, respectively, but had no effects on seedhead production 5 and 7 WAT (Table 3.25). The bacterium in combination with Rewet at 16.10, 22.54, and 32.20 L ha^{-1} suppressed seedhead production by 27, 16, and 30% 3 WAT, but had no effect on seedhead production at 5 and 7 WAT (Table 3.25). Treatment combinations of Rely and Rewet with *Xanthomonas campestris* did not affect the growth or quality of annual bluegrass or creeping bentgrass (Table 3.23, Table 3.24, and Table 3.26).

Discussion

Xanthomonas campestris suppressed both biotypes of annual bluegrass by over 90% in growth room experiments, yet caused no reductions in annual bluegrass encroachment in field trials. This is consistent with the findings of Zhou and Neal (1995)

who concluded that failure of the bacterium to control annual bluegrass in the field could be attributed to climatic conditions unsuitable for the bacterium in the northeastern U.S.

In growth room experiments, surfactants when combined with the bacterial suspension did not improve the activity of *Xanthomonas campestris* against annual bluegrass. Effects of surfactants on suppression of annual bluegrass by *Xanthomonas campestris* were either non-significant or inhibitory. Rely and Rewet had no influence on activity of the bacterium at any of the rates tested, with regards to annual bluegrass growth, quality, or seedhead production, or the quality of creeping bentgrass. The same is true for Break-thru treatment at the lowest rate [0.3% (V/V)].

Tank-mix combination with Agri-dex at 0.5% (V/V) did not improve suppression of annual bluegrass, but reduced clipping fresh weight of the annual biotype of annual bluegrass by over 50% 2 WAT compared to the bacterium alone. This treatment had no adverse effects on quality or growth of creeping bentgrass.

Silwet L-77, X-77, and the higher rates of Break-thru, were antagonistic to the activity of the bacterium, particularly in the case of the perennial biotype of annual bluegrass. Suppression of the perennial biotype was reduced by Silwett L-77, X-77, and Break-thru, by 23, 35, and 87% 7 WAT, respectively, compared to the bacterium alone. Imaizumi and Fujimori (1997) suggested that competition between plant growth and bacterial multiplication dictates the level of disease severity, and the differential response between biotypes could be attributed to the more aggressive nature of the perennial biotypes of annual bluegrass in that respect. Silwett L-77, X-77, and Break-thru were toxic to *Xanthomonas campestris* at concentrations above 0.1% (V/V) in amended media

studies (Chapter II). Antagonistic effects of the surfactants may have been more pronounced in the perennial biotype of annual bluegrass because the higher internal populations of *Xanthomonas campestris* required for control of the perennial biotypes are not established under exposure to toxic levels of the surfactants.

Treatments of Silwet L-77, X-77, and the higher rates of Break-thru resulted in temporary injury (yellowing) of creeping bentgrass in growth room and field experiments. The injured creeping bentgrass recovered shortly after applications ceased.

In summary, none of the surfactants tested improved the activity of *Xanthomonas campestris* in the suppression of annual bluegrass. Agri-dex accelerated the infection of the annual biotype of annual bluegrass early in the course of the experiments, but had no effect in the end. Similarly, Rely and Rewet suppressed seedhead formation early on (3 WAT). Otherwise, treatment combinations of Rely, Rewet, and the lowest rate of Break-thru with the bacterium had no effects on the activity of *Xanthomonas campestris*. Silwet L-77, X-77, and high rates [0.6 and 0.9% (V/V)] of Break-thru inhibited the activity of the bacterium and caused temporary damage to creeping bentgrass.

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CHAPTER IV

EFFECTS OF PLANT GROWTH REGULATORS ON THE ACTIVITY OF *XANTHOMONAS CAMPESTRIS* PV. *POANNUA* IN ANNUAL BLUEGRASS SUPPRESSION

Abstract

Growth room and field experiments were conducted to determine the effects of various plant growth regulators on activity of *Xanthomonas campestris* pv. *poannua* in the suppression of two biotypes of annual bluegrass (*Poa annual* L.). Effects of plant growth regulators and the bacterium on creeping bentgrass (*Agrostis palustris* Huds.) were also examined. *Xanthomonas campestris* at 10^7 cfu ml^{-1} suppressed both biotypes of annual bluegrass by over 90% in the growth room, yet did not suppress annual bluegrass in field trials, either applied alone or in combination with growth regulators. Paclobutrazol and ECSS 1001 did not affect annual bluegrass suppression by *Xanthomonas campestris*. The combination of trinexapac-ethyl and *Xanthomonas campestris* reduced suppression of the perennial biotype of annual bluegrass in the growth room by 50% 7 WAT, compared to the bacterium alone. Rapid reductions in growth (74% 3 DAT) of annual bluegrass caused by trinexapac-ethyl may have resulted in inadequate wounding for infection by *Xanthomonas campestris*. Trinexapac-ethyl and paclobutrazol, increased seedhead production of annual bluegrass by over 20% 5 WAT in field trials. Annual bluegrass populations in a putting green were not reduced and creeping bentgrass was not injured by any of the treatments.

Introduction

Xanthomonas campestris pv. *poannua* controls annual bluegrass by causing a vascular wilt that leads to plant death (Imaizumi et al., 1997; Johnson, 1994; Nishino et al., 1995). The perennial biotypes of annual bluegrass [*Poa annua* var. *reptans* (Hauskins) Timm], which are the dominant type infesting putting greens in the northern U.S., are less susceptible to the disease than their annual counterpart (*Poa annua* var. *annua*) (Zhou and Neal 1995). Imaizumi and Fujimori (1997) observed that the perennial biotype of annual bluegrass plants continued to grow and produced tillers, even when infected with bacterial populations that killed the annual biotype. They concluded that competition between plant growth and bacterial multiplication dictates the level of disease severity, and the differential response between biotypes could be attributed to the more aggressive nature of the perennial biotypes of annual bluegrass in that respect.

Although chemical control of annual bluegrass has been inconsistent (Watschke et al., 1979; Gaul and Christians, 1988), turf managers often employ plant growth regulators as part of an integrated approach to management of annual bluegrass. Plant growth regulators such as amidochlor [*N*-[(acetylamino)methyl]-2-chloro-*N*-2,6(diethylphenyl)acetamide]are known to reduce shoot growth and seedhead production in Kentucky bluegrass (*Poa pratensis* L.) and red fescue (*Festuca rubra* L.) (Bhowmik, 1987). Annual bluegrass growth and seedhead suppression has been accomplished with various plant growth regulators including paclobutrazol [(±)-(R*,R*)-§-[(4-chlorophenol) methyl]-a-(1,1 dimethyl)-1H-1,2,4-triazole-1-ethanol], trinexapac-ethyl [4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethyl ester], and

melfluidide [*N*-[2, 4-dimethyl-5-[[(trifluoromethyl)sulfonyl] amino]phenyl] acetamide] (Fagerness and Penner, 1998; Watschke and Borger, 1998).

Treatments with plant growth regulators might suppress the aggressive nature of the perennial biotype of annual bluegrass that may enable the plant to endure infection by *Xanthomonas campestris*. Such inhibition might therefore make the perennial biotype of annual bluegrass more susceptible to the vascular wilt caused by the bacterium.

The objectives of our experiments were to a) determine the effects of three plant growth regulators on the activity of *Xanthomonas campestris* in suppressing growth or seedhead production of annual bluegrass and b) determine the effects of *Xanthomonas campestris* and plant growth regulators on the quality of creeping bentgrass in a putting green.

Materials and methods

Growth room experiment

Annual bluegrass and creeping bentgrass were grown side by side in 11.4 cm pots. Seeds of an annual biotype of annual bluegrass and 'G2'creeping bentgrass were sown at 152.5 $kg \cdot ha^{-1}$ and 76 $kg \cdot ha^{-1}$, respectively. Plugs of a perennial biotype of annual bluegrass measuring 5.1 cm in diam. by 5.1 cm deep were collected from a putting green at the Turf Research Center in South Deerfield, MA. The plugs were transplanted into 10.2 cm diam. pots. The soil was a Hadley fine sandy loam (Typic Udifluvents) containing 3.5% organic matter, with a pH of 6.5. The soil was autoclaved prior to

seeding. The growth chamber had an average temperature of 24 C (± 2 C) and relative humidity of 32% (± 3%). Fluorescent lighting was used in a 16 h cycle. The plants were watered and fertilized as needed to avoid water or nutritional stress, and clipped twice weekly to a height of 2.5 cm.

Trinexapac-ethyl, paclobutrazol and ECSS 1001 (an experimental plant growth regulator) were used at their recommended rates, each alone, and each in combination with *Xanthomonas campestris*. Trinexapac-ethyl and ECSS-1001 were applied at 0.43 L ha^{-1} and 3.43 g ha^{-1}, respectively, using a hand pump sprayer. Paclobutrazol (G) was applied at 85 kg ha^{-1} (358 g a.i. ha^{-1}) containing 31% N. Growth regulators were applied separately from the bacterium on June 2, 2000, the first of five weekly bacterium applications. *Xanthomonas campestris* was applied as a prepared liquid culture containing 10^9 colony forming units per milliliter (cfu ml^{-1}), at 9.46 L·ha^{-1}. All treatments were applied at a spray volume of 946 L·ha^{-1}, using a hand pump sprayer. Within five minutes of each treatment application, plants were clipped to a height of 2.5 cm. Other treatments included the bacterium alone and an untreated control. All treatments were applied to both annual and perennial types of annual bluegrass, and creeping bentgrass. All treatments were replicated four times in a completely randomized block design.

Visual ratings

Quality of annual bluegrass and creeping bentgrass, and seedhead production by annual bluegrass were evaluated by visual ratings. Ratings were made weekly throughout the course of the experiment. Annual bluegrass suppression was determined at 3, 4 and 7 weeks after the first treatment (WAT). Visual ratings of both annual and perennial types of annual bluegrass, and creeping bentgrass were estimated based on a 1 to 9 scale, where 1 = dead grass and 9 = healthy grass. Seedhead production of the perennial type annual bluegrass was estimated based on a scale of 0 to 100%, where 0 = no seedheads were produced and 100 = all shoots produced seedheads.

Clipping fresh weights

All plants were clipped twice a week, once immediately following treatment and once three days later, at which time clipping weights were recorded. The clipping weight of each treatment was converted to a percent of the clipping weight of the untreated control.

Experimental design

Both experiments were arranged in a completely randomized block design. Analysis of variance (ANOVA) was used to determine differences in treatment effects. Error terms were calculated using the expected mean squares (Damon and Harvey, 1987). Hypothesis tests were performed with the appropriate error terms by using the general linear model program of SAS (SAS Institute, 1995). Predetermined linear contrasts were used to test for significant differences between specific treatments (Table 4.1). The untreated control and the treatment receiving the bacterium alone were compared. The treatment receiving the bacterium alone was also compared separately with all other treatments. Each growth regulator alone was compared with the corresponding combination of growth regulator plus the bacterium. F values with probabilities equal to or less than 0.05 were considered significant, and designated by an "*". F values of equal to or less than 0.01 were considered highly significant and designated by a "**".

Table 4.1. Sources of variation from the ANOVA for the effects of various treatment combinations of *Xanthomonas campestris* pv. *poannua* and plant growth regulators on annual bluegrass and creeping bentgrass in growth room and field experiments.

Source of variation	df
Corrected total	31
Block	3
Treatment	7
Linear contrasts:	
Untreated control vs. *Xanthomonas campestris* alone	1[a]
Xanthomonas campestris alone vs. *Xanthomonas campestris* + trinexapac-ethyl	1
Xanthomonas campestris alone vs. *Xanthomonas campestris* + paclobutrazol	1
Xanthomonas campestris alone vs. *Xanthomonas campestris* + ECSS 1001	1
Trinexapac-ethyl alone vs. Trinexapac-ethyl + *Xanthomonas campestris*	1
Paclobutrazol alone vs. Paclobutrazol + *Xanthomonas campestris*	1
ECSS 1001 alone vs. ECSS 1001 + *Xanthomonas campestris*	1
Error	21

[a] Contrast not used on clipping fresh weight data.

Field experiment

The field experiment was conducted at the Turf Research Center in South Deerfield. The soil was composed of sand and native Winooski soil, with a pH of 6.0. Plots were laid out on a putting green of established 'Penncross' creeping bentgrass, heavily infested with a perennial biotype of annual bluegrass. The putting green was mowed three times every week at a height of 0.635 cm, including once for treatment applications. The putting green was maintained with normal irrigation and disease management practices that excluded the use of systemic fungicides. Fertility was monitored closely, and nitrogen was applied every two weeks at 12.7 kg ha^{-1}.

Treatment applications were made after 4:30 pm to minimize the effects of UV light on the bacterium. Treatments were applied using an 11.4 L compressed air sprayer with a spray volume of 946 L·ha^{-1}. To protect the bacterium against desication, treated plots were mowed within five minutes of spraying using a Jacobsen walk behind greens mower.

Trinexapac-ethyl, paclobutrazol, and ECSS 1001 were used in combination with *Xanthomonas campestris*. Trinexapac-ethyl and ECSS-1001 were applied at 0.43 L a.i.·ha^{-1} and 3.43 g·ha^{-1}, respectively, using an 11.4 L compressed air sprayer. Paclobutrazol (G) was applied at 85 kg ha^{-1} (358 g a.i. ha^{-1}) containing 31% N. The bacterium was applied five times in weekly intervals during the month of May, 2000. On each occasion prepared liquid culture of *Xanthomonas campestris* containing 10^9 cfu ml^{-1} was applied at 9.46 L·ha^{-1} and the spray volume of 946 L·ha^{-1}, using an 11.4 L compressed air sprayer. Other treatments included the bacterium alone and an untreated

control. All treatments were replicated six times in a non-randomized (due to the mowing requirement) complete block design.

Visual ratings

Visual ratings of annual bluegrass cover, seedhead production, and visual quality were recorded periodically beginning at the onset of the experiment and ending 14 weeks after the first application (WAT). Creeping bentgrass quality was visually rated periodically from 3 WAT to 14 WAT. Annual bluegrass cover was estimated as the percentage of plot area covered by annual bluegrass. Ratings of annual bluegrass cover made after the first rating were converted to change in annual bluegrass cover by subtracting the initial week's rating. Seedhead production was recorded as a percentage, where 0 = none of the annual bluegrass shoots had seedheads and 100% = all of the annual bluegrass shoots had seedheads. Annual bluegrass quality ratings were made on a scale from 1 to 9, where 1 = dead and 9 = healthy. Creeping bentgrass quality ratings were made on a 1 to 9 scale, where 1 = dead grass and 9 = a healthy, dense, uniform stand of turfgrass. Ratings were made with consistency regarding time of day and time after mowing.

Experimental design

All data were subjected to analysis of variance (ANOVA) to determine differences in treatment effects. Error terms were calculated using the expected mean squares (Damon and Harvey, 1987). Hypothesis tests were performed with the

appropriate error terms by using the general linear model program of SAS (SAS Institute, 1995). Linear contrasts were used to test for significant differences between specific treatments (Table 4.1). The untreated control and the treatment receiving the bacterium alone were compared. The treatment receiving the bacterium alone was also compared separately with all other treatments. Each growth regulator alone was compared with the corresponding combination of growth regulator plus the bacterium. F values with probabilities equal to or less than 0.05 were considered significant, and designated by an "*". F values of equal to or less than 0.01 were considered highly significant and designated by a "**".

Results

Growth room experiment

Xanthomonas campestris applied at 9.46 L ha^{-1} suppressed the annual biotype of annual bluegrass by 92% (Table 4.2 and Figure 4.1), while it suppressed the perennial biotype by 100% (Table 4.3) 7 WAT. Clipping fresh weights of annual and perennial biotypes of annual bluegrass were reduced by 99 and 89% 4 WAT, respectively (Table 4.4, Table 4.5 and Figure 4.2). Quality of both biotypes was reduced by the bacterium by over 90% 4 WAT (Table 4.7 and Table 4.8). Neither clipping fresh weight (Table 4.6) nor quality (Table 4.9) of creeping bentgrass was affected by *Xanthomonas campestris.* Seedhead production by annual bluegrass was not affected by any of the treatments (Table A.9).

Table 4.2. Suppression of an annual biotype of annual bluegrass with *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various plant growth regulators.

Treatment	Rate L or g ha^{-1}	Percent weed suppression, WAT[a] 3	4	7
Untreated control		0.00	0.00	0.00
Xanthomonas campestris[b] alone	9.46 L	90.00	99.75	92.50
Trinexapac-ethyl alone	0.43 L	0.00	0.00	0.00
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L + 9.46 L	77.50	91.25	95.00
Paclobutrazol alone	358.00 g	0.00	0.00	0.00
Paclobutrazol + *Xanthomonas campestris*	358.00 g + 9.46 L	95.75	93.50	93.75
ECSS 1001 alone	3.43 g	20.00	22.50	17.50
ECSS 1001 + *Xanthomonas campestris*	3.43 g + 9.46 L	86.25	95.00	97.50
F-value for 'Treatment'		**	**	**

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

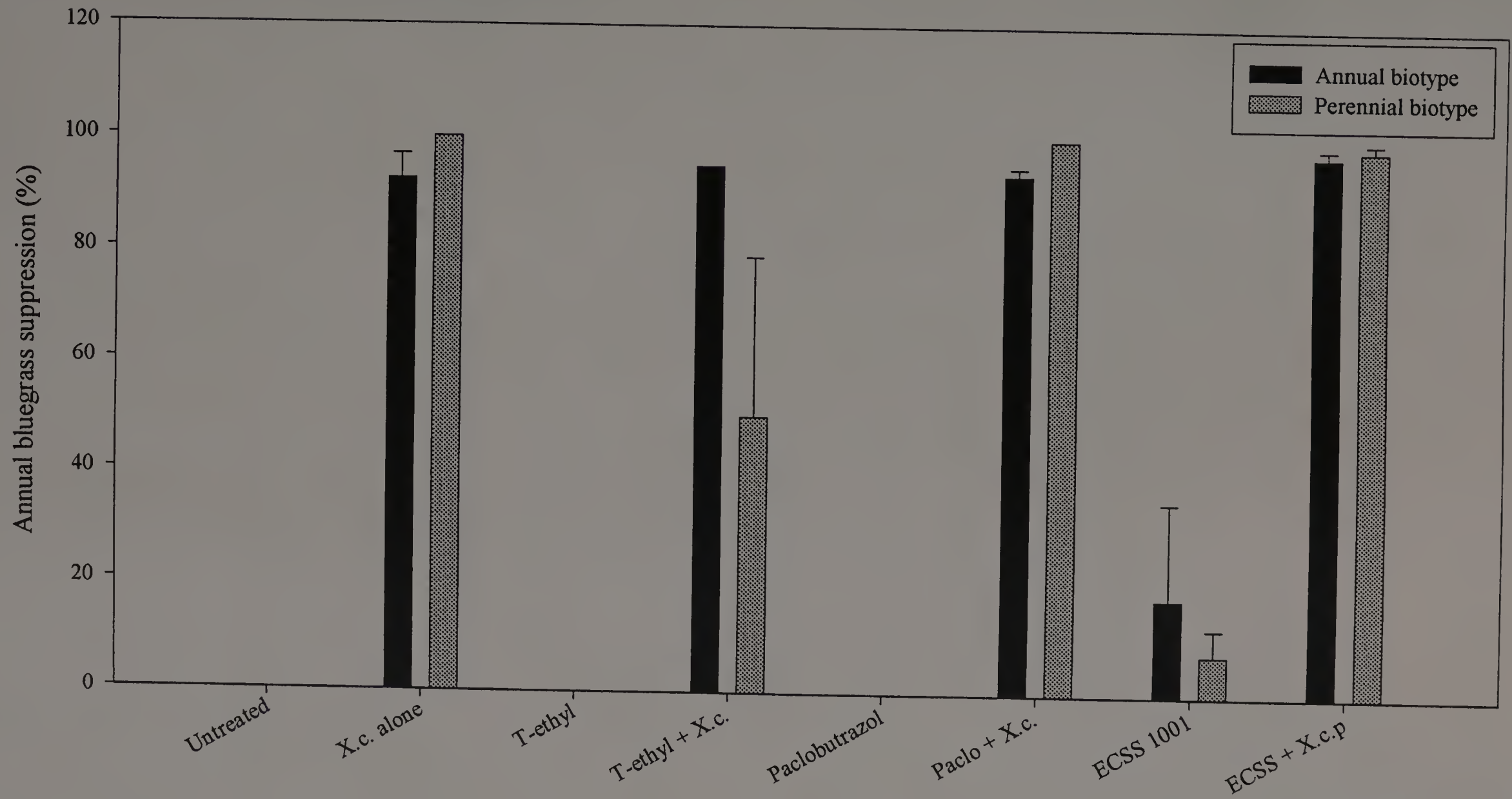

Figure 4.1. Suppression of two biotypes of annual bluegrass with *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various plant growth regulators 7 WAT.

Table 4.3. Suppression of a perennial biotype of annual bluegrass with *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various plant growth regulators.

Treatment	Rate L or g ha^{-1}	Percent weed suppression, WAT[a] 3	4	7
Untreated control		0.00	0.00	0.00
Xanthomonas campestris[b] alone	9.46 L	66.25	96.00	100.00
Trinexapac-ethyl alone	0.43 L	0.00	0.00	0.00
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	37.50	48.50	50.00
Paclobutrazol alone	358.00 g	0.00	0.00	0.00
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	98.25	97.25	100.00
ECSS 1001 alone	3.43 g	1.25	0.00	7.50
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	93.25	92.50	98.75
F-value for 'Treatment'		**	**	**

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

Table 4.4. Clipping fresh weight of an annual biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Clipping fresh weight (% of untreated control), WAT[a] 0	1	2	3	4
Xanthomonas campestris[b] alone	9.46 L	93.93	90.57	48.42	14.05	0.60
Trinexapac-ethyl alone	0.43 L	48.83	40.79	30.37	89.97	107.91
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	43.29	36.83	29.70	25.54	12.73
Paclobutrazol alone	358.00 g	101.97	45.95	18.98	21.68	18.51
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	97.31	38.36	1.73	0.00	0.78
ECSS 1001 alone	3.43 g	87.51	74.81	105.43	114.25	130.46
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	90.89	87.68	39.73	13.68	2.38
F-value for 'Treatment'		**	**	**	**	**

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

Table 4.5. Clipping fresh weight of a perennial biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Clipping fresh weight (% of untreated control), WAT[a] 0	1	2	3	4
Xanthomonas campestris[b] alone	9.46 L	98.48	55.49	26.19	17.77	10.61
Trinexapac-ethyl alone	0.43 L	26.84	12.80	9.97	32.62	64.93
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	25.72	8.28	28.47	31.00	20.83
Paclobutrazol alone	358.00 g	82.26	42.55	16.11	19.10	28.50
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	76.26	23.19	3.79	0.00	0.00
ECSS 1001 alone	3.43 g	129.32	64.05	97.71	105.16	133.65
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	142.53	64.59	25.69	11.27	19.10
F-value for 'Treatment'		**	**	**	**	**

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

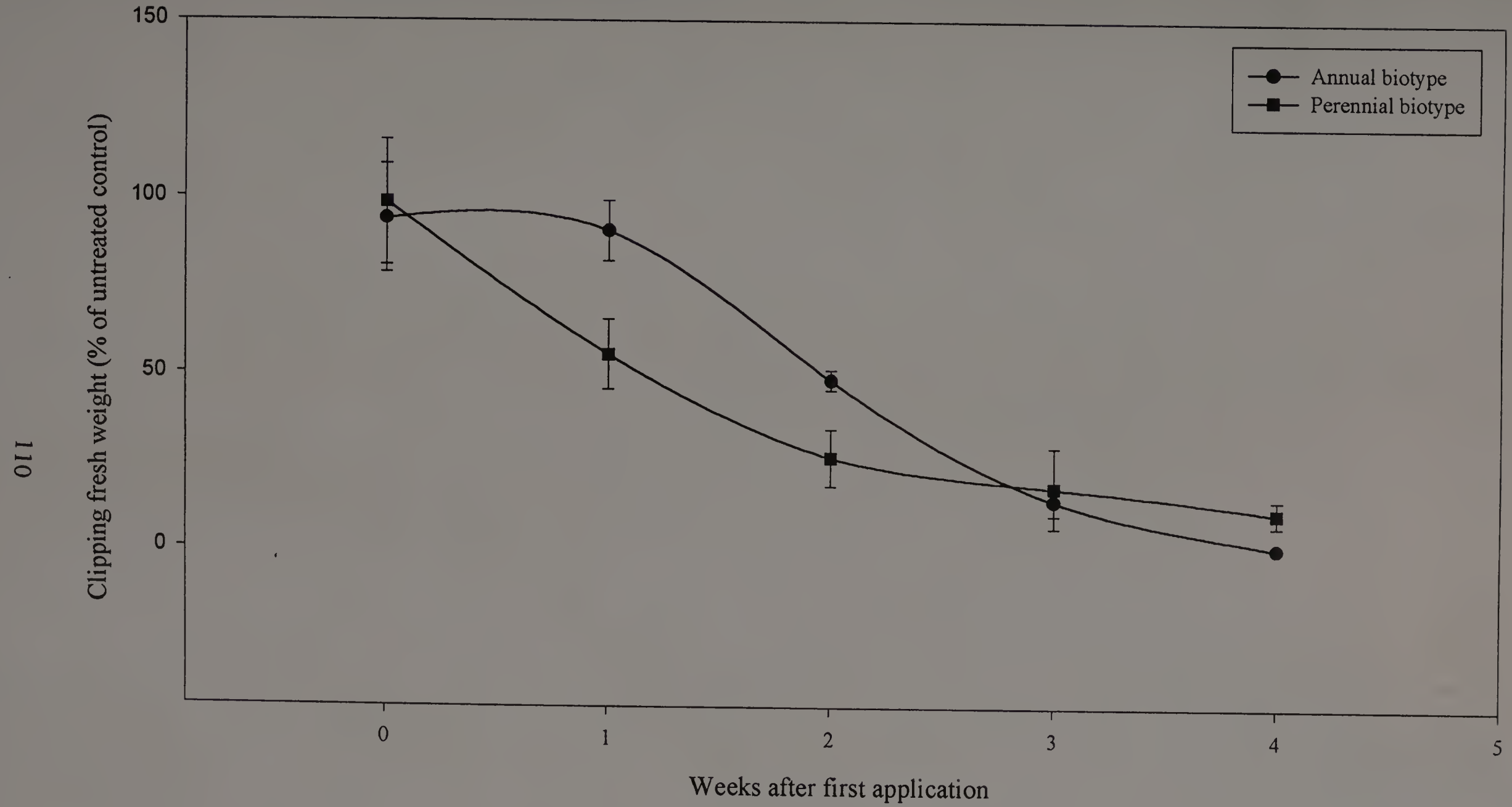

Figure 4.2. Effects of *Xanthomonas campestris* pv. *poannua* on clipping fresh weights of two biotypes of annual bluegrass under controlled conditions.

Table 4.6. Clipping fresh weight of 'G2' creeping bentgrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Clipping fresh weight (% of untreated control), WAT[a] 0	1	2	3	4
Xanthomonas campestris[b] alone	9.46 L	89.95	106.93	83.84	122.83	156.22
Trinexapac-ethyl alone	0.43 L	46.63	24.52	17.34	110.31	128.09
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	32.58	17.71	14.66	119.14	179.16
Paclobutrazol alone	358.00 g	77.76	29.41	3.54	7.23	55.79
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	86.49	27.85	8.14	22.30	42.87
ECSS 1001 alone	3.43 g	83.91	75.67	74.04	101.58	122.61
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	93.66	84.24	80.40	108.33	147.25
F-value for 'Treatment'		**	**	**	**	*

[a] WAT = weeks after first application.
[b] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

Table 4.7. Visual quality of an annual biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Turfgrass quality rating[a], WAT[b] 0	1	2	3	4
Untreated control		9.00	9.00	9.00	9.00	9.00
Xanthomonas campestris[c] alone	9.46 L	9.00	8.75	8.00	2.00	1.25
Trinexapac-ethyl alone	0.43 L	9.00	9.00	9.00	9.00	9.00
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	9.00	9.00	7.00	2.50	2.00
Paclobutrazol alone	358.00 g	9.00	9.00	9.00	9.00	9.00
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	9.00	9.00	5.25	2.00	2.00
ECSS 1001 alone	3.43 g	9.00	9.00	8.25	7.25	7.50
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	9.00	9.00	5.00	2.00	1.50
F-value for 'Treatment'		ns	ns	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

Table 4.8. Visual quality of a perennial biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Turfgrass quality rating[a], WAT[b] 0	1	2	3	4
Untreated control		9.00	9.00	9.00	9.00	9.00
Xanthomonas campestris[c] alone	9.46 L	9.00	8.50	7.00	2.75	1.50
Trinexapac-ethyl alone	0.43 L	9.00	8.50	6.50	5.00	6.75
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	8.25	7.50	6.25	5.50	4.00
Paclobutrazol alone	358.00 g	8.75	7.75	8.00	7.75	8.00
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	9.00	8.50	6.00	1.75	1.50
ECSS 1001 alone	3.43 g	8.50	8.50	8.75	8.00	8.25
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	8.25	8.25	7.50	2.00	1.50
F-value for 'Treatment'		ns	ns	ns	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}

Table 4.9. Visual quality of 'G2' creeping bentgrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Turfgrass quality rating[a], WAT[b] 0	1	2	3	4
Untreated control		9.00	9.00	9.00	9.00	9.00
Xanthomonas campestris[c] alone	9.46 L	9.00	9.00	9.00	9.00	9.00
Trinexapac-ethyl alone	0.43 L	9.00	9.00	7.75	8.00	9.00
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	9.00	9.00	8.25	8.00	9.00
Paclobutrazol alone	358.00 g	9.00	9.00	8.00	8.00	9.00
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	9.00	9.00	8.00	8.00	9.00
ECSS 1001 alone	3.43 g	9.00	9.00	9.00	9.00	9.00
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	9.00	9.00	9.00	9.00	9.00
F-value for 'Treatment'		ns	ns	**	**	ns

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}

Trinexapac-ethyl alone at 0.43 L ha^{-1} did not suppress annual bluegrass (Figure 4.1) in the growth room. Clipping fresh weights of annual (Table 4.4) and perennial (Table 4.5) biotypes of annual bluegrass were reduced by 70 and 90% 1 WAT, while the clipping fresh weight of creeping bentgrass (Table 4.6) was reduced by 83% 1 WAT.

The combination of *Xanthomonas campestris* and trinexapac-ethyl at 0.43 L ha^{-1} reduced suppression of the perennial biotype of annual bluegrass by 50% 7 WAT compared to that of the bacterium alone (Table 4.3). In contrast, the same treatment had no effect on suppression of the annual biotype of annual bluegrass (Table 4.2 and Figure 4.1). The combination of the bacterium and trinexapac-ethyl at 0.43 L ha^{-1} resulted in significant reductions in clipping fresh weight of both biotypes of annual bluegrass up to 2 WAT (Figure 4.3 and Figure 4.4). Compared to *Xanthomonas campestris* alone, the combination of the bacterium and trinexapac-ethyl had no effect on the quality of the annual biotype of annual bluegrass (Table 4.7), but improved the quality of the perennial biotype by over 30% 3 and 4 WAT (Table 4.8). The combination of trinexapac-ethyl and the bacterium had no effects on clipping fresh weight (Table 4.6) or quality (Table 4.9) of creeping bentgrass in the growth room, as compared to trinexapac-ethyl alone.

The combination of *Xanthomonas campestris* and paclobutrazol at 358 g ha^{-1} increased suppression of the perennial biotype of annual bluegrass by 32% 3 WAT (Table 4.3 and Figure 4.5), as compared to the bacterium alone, but had no effect after that time period. Paclobutrazol plus *Xanthomonas campestris* did not affect suppression of the annual biotype of annual bluegrass compared to the bacterium alone (Table A.1 and Figure 4.5). Addition of paclobutrazol reduced clipping fresh weights of the annual

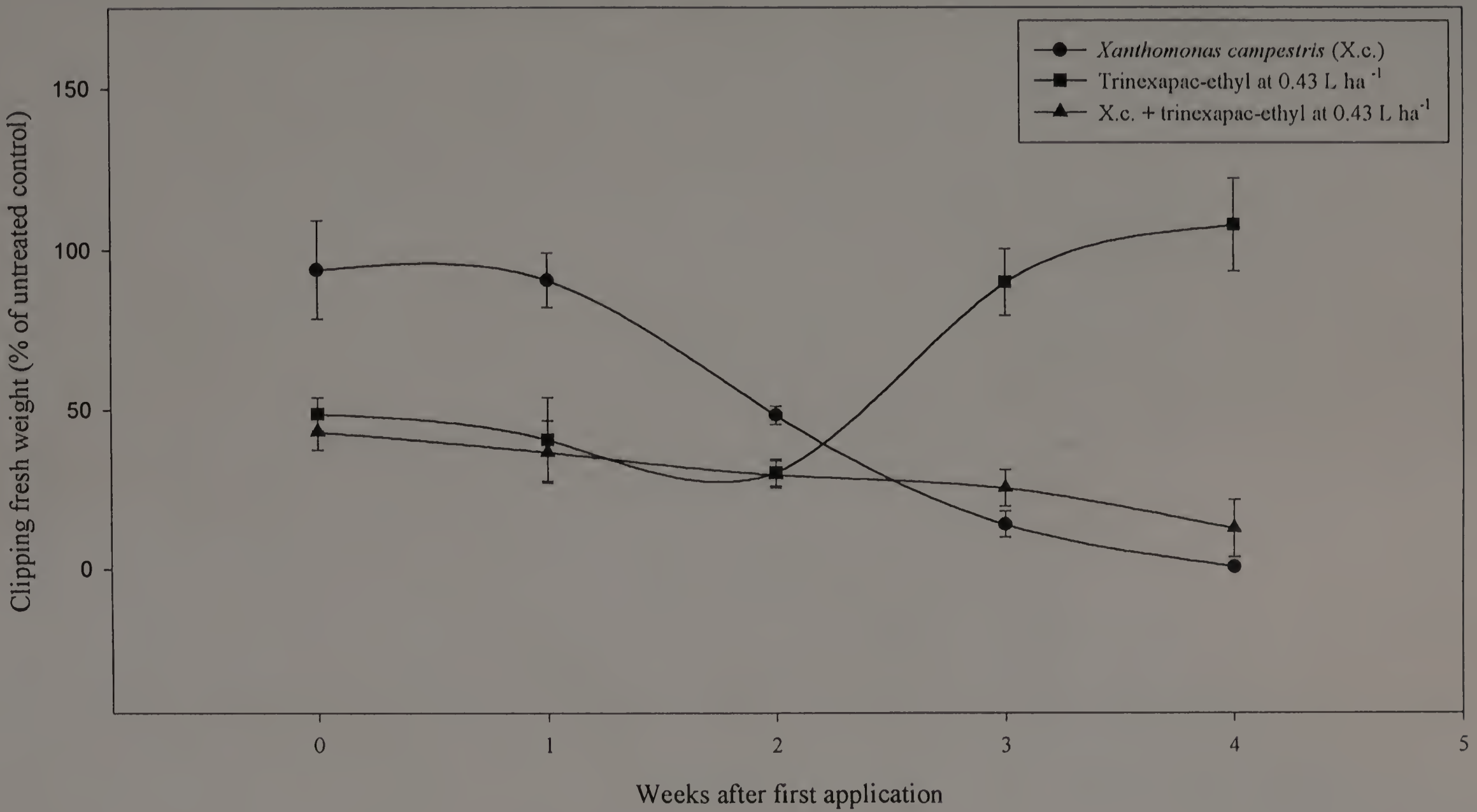

Figure 4.3. Effects of *Xanthomonas campestris* pv. *poannua*, alone or in combination with trinexapac-ethyl, on clipping fresh weight of an annual biotype of annual bluegrass under controlled conditions.

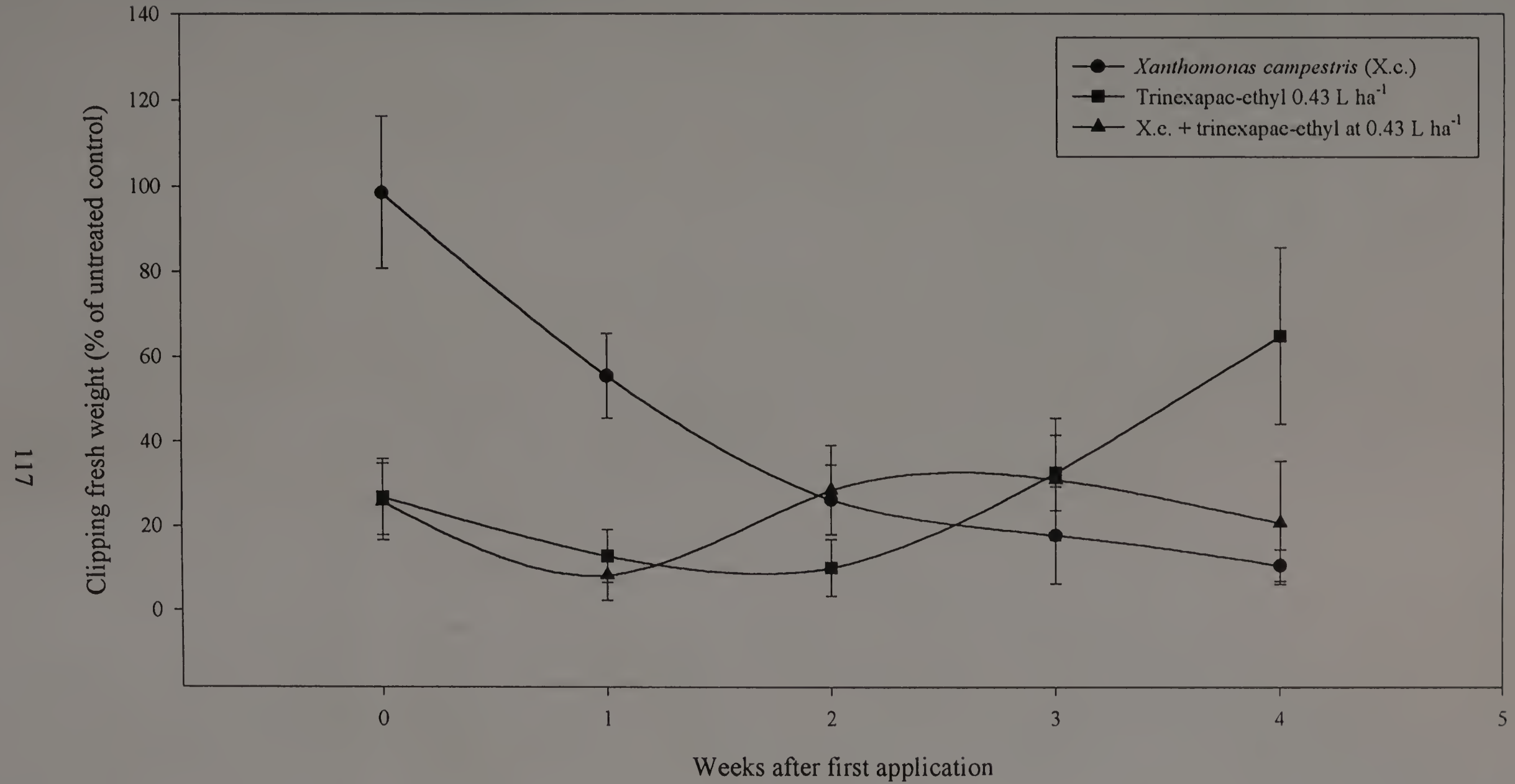

Figure 4.4. Effects of *Xanthomonas campestris* pv. *poannua,* alone or in combination with trinexapac-ethyl on clipping fresh weight of a perennial biotype of annual bluegrass.

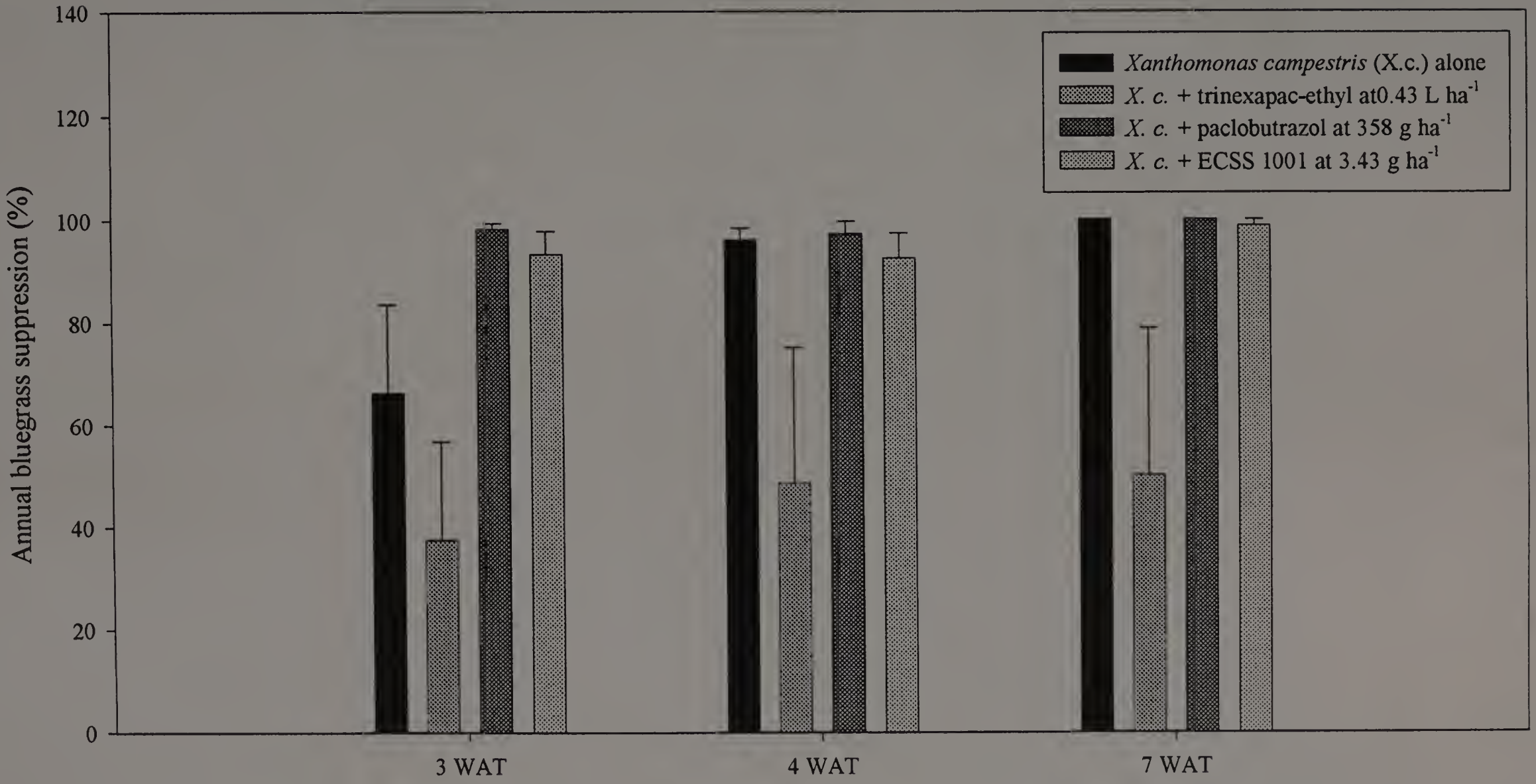

Figure 4.5. Suppression of a perennial biotype of annual bluegrass by *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various plant growth regulators.

biotype of annual bluegrass by over 40% 1 and 2 WAT (Table 4.4 and Figure 4.6), and the perennial biotype by 32% 1 WAT (Table 4.5 and Figure 4.7), as compared to the bacterium alone. The same treatment reduced the quality of the annual biotype of annual bluegrass by 21% 2 WAT (Table 4.7), but did not reduce the quality of the perennial biotype (Table A.7). Paclobutrazol, when combined with the bacterium, had no effect on creeping bentgrass quality (Table A.8) or clipping fresh weight (Table A.5), as compared to paclobutrazol alone.

ECSS 1001 alone suppressed annual (Table 4.2) and perennial (Table 4.3) biotypes of annual bluegrass by 17 and 7% 7 WAT, respectively, in the growth room (Figure 4.1). In combination with *Xanthomonas campestris*, ECSS 1001 suppressed the perennial biotype of annual bluegrass by 27% more than the bacterium alone 3 WAT (Table 4.3), but did not improve suppression of the perennial biotype (Table A.2 and Figure 4.1). Clipping fresh weights of either annual or perennial biotypes of annual bluegrass were not affected by ECSS 1001 compared to the bacterium alone (Table A.3 and Table A.4). The combination of *Xanthomonas campestris* and ECSS 1001 reduced the quality of the annual biotype of annual bluegrass by 37% 2 WAT (Table 4.7), but had no effect on the quality of the perennial biotype (Table A.7).

Field experiment

Xanthomonas campestris alone did not suppress infestation (Table A.10 and Figure 4.8), quality (Table A.11) or seedhead production (Table A.12) of annual bluegrass, or the quality of creeping bentgrass (Table A.13).

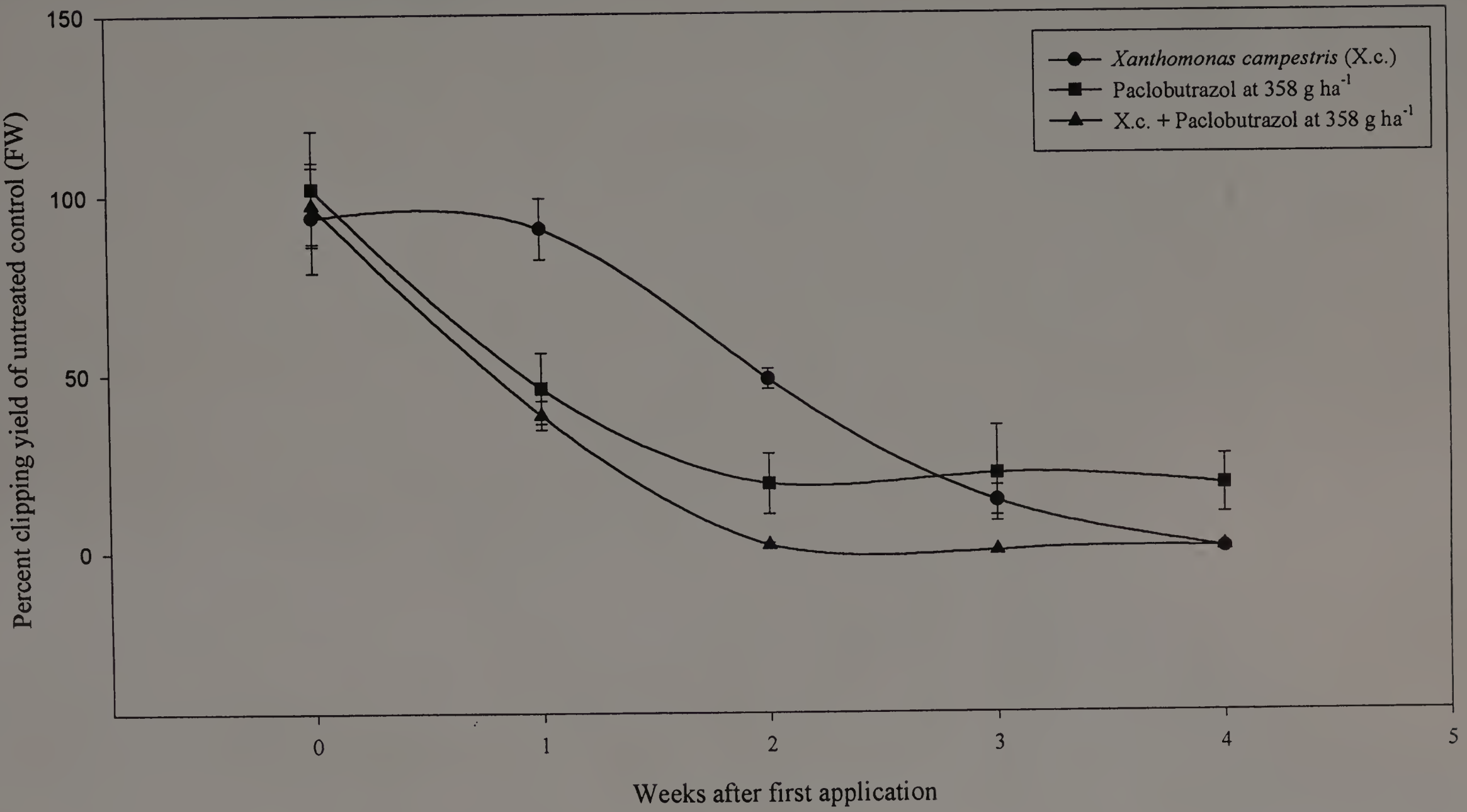

Figure 4.6. Effects of *Xanthomonas campestris* pv. *poannua*, alone or in combination with paclobutrazol on clipping freshweight of an annual biotype of annual bluegrass under controlled conditions.

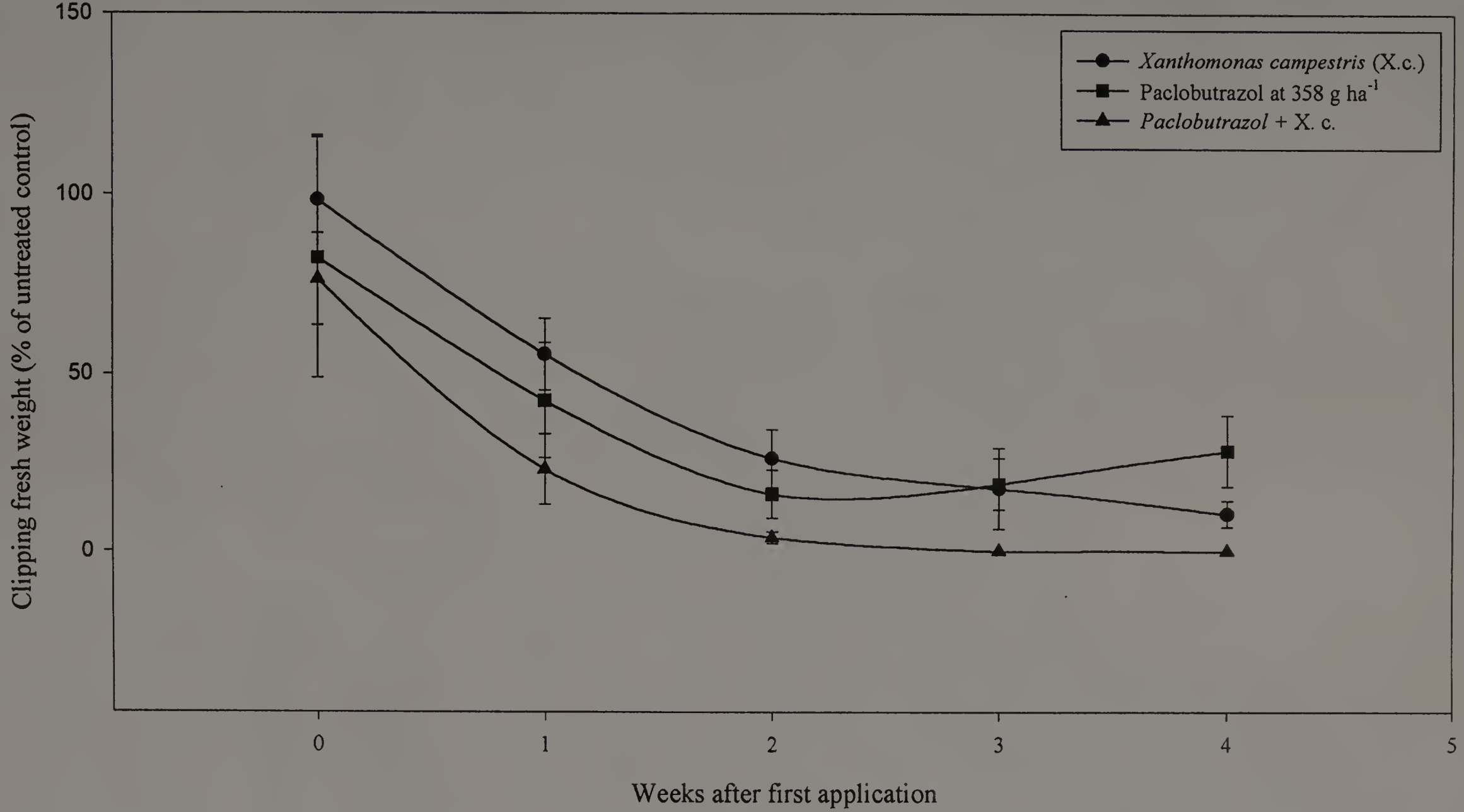

Figure 4.7. Effects of *Xanthomonas campestris* pv. *poannua*, alone or in combination with paclobutrazol on clipping fresh weight of a perennial biotype of annual bluegrass.

Trinexapac-ethyl in combination with *Xanthomonas campestris* reduced annual bluegrass encroachment by 5% 4 WAT and 7 WAT, compared to the bacterium alone (Table 4.10), but had no effect at 9 WAT (Figure 4.8). Seedhead production was increased by trinexapac-ethyl applied alone and in combination with the bacterium by over 20% 7 WAT (Table 4.12). The combination of the *Xanthomonas campestris* and trinexapac-ethyl did not affect the quality of annual bluegrass (Table 4.11) or creeping bentgrass (Table 4.13). Annual bluegrass infestation (Table A.10), quality (Table A.11), and seedhead production (Table A.12), as well as quality of creeping bentgrass (Table A.13), were unaffected by the combination of *Xanthomonas campestris* and trinexapac-ethyl, as compared to trinexapac-ethyl alone.

Paclobutrazol in combination with *Xanthomonas campestris* reduced the spread of annual bluegrass by 5% 4, 7, and 9 WAT, compared to the bacterium alone (Table 4.10 and Figure 4.8). The addition of paclobutrazol reduced quality of annual bluegrass by over 40% 5, 7, and 9 WAT (Table 4.11 and Figure 4.9) and increased seedhead production by 30% 7 WAT, compared to the bacterium alone (Table 4.12). The combination of *Xanthomonas campestris* and paclobutrazol had no effect on the quality of creeping bentgrass, as compared to the bacterium treatment alone (Table 4.13). Annual bluegrass encroachment (Table A.10), quality (Table A.11), and seedhead production (Table A.12), as well as quality of creeping bentgrass (Table A.13), were unaffected by the combination of *Xanthomonas campestris* and paclobutrazol, as compared to paclobutrazol alone.

Table 4.10. Annual bluegrass encroachment on a 'Penncross' creeping bentgrass putting green as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Percent annual bluegrass increase[a], WAT[b] 3	4	5	7	9
Untreated control		11.67	15.00	7.50	13.33	13.33
Xanthomonas campestris[c] alone	9.46 L	10.83	10.83	9.17	14.17	12.50
Trinexapac-ethyl alone	0.43 L	5.00	3.33	0.83	8.33	6.67
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	7.50	5.00	4.17	8.33	7.50
Paclobutrazol alone	358.00 g	10.00	10.83	4.17	10.33	7.50
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	8.33	8.33	5.83	10.00	5.83
ECSS 1001 alone	3.43 g	3.33	4.17	3.33	4.17	3.33
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	1.67	7.50	2.50	3.33	2.50
F-value for 'Treatment'		**	**	ns	**	*

[a] Cumulative increase in plot area covered by annual bluegrass compared to 0 WAT.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}

Table 4.11. Visual quality of annual bluegrass in a 'Penncross' creeping bentgrass putting green as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Turfgrass quality rating[a], WAT[b] 3	4	5	7	9
Untreated control		9.00	9.00	9.00	9.00	9.00
Xanthomonas campestris[c] alone	9.46 L	9.00	9.00	9.00	7.83	8.50
Trinexapac-ethyl alone	0.43 L	8.00	9.00	9.00	8.50	9.00
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	8.00	9.00	9.00	8.33	9.00
Paclobutrazol alone	358.00 g	9.00	7.50	4.00	5.50	5.33
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	9.00	7.50	5.33	5.67	5.17
ECSS 1001 alone	3.43 g	9.00	9.00	7.83	7.50	7.67
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	9.00	9.00	8.33	7.67	7.67
F-value for 'Treatment'		**	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

Table 4.12. Annual bluegrass seedhead production on a 'Penncross' creeping bentgrass putting green as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Percent seedhead production[a], WAT[b] 3	5	7
Untreated control		70.00	70.83	64.17
Xanthomonas campestris[c] alone	9.46 L	69.17	77.50	62.50
Trinexapac-ethyl alone	0.43 L	79.17	75.83	90.83
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	80.83	80.00	88.33
Paclobutrazol alone	358.00 g	86.67	63.33	94.17
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	84.17	64.17	95.00
ECSS 1001 alone	3.43 g	72.50	62.50	70.00
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	70.83	66.67	70.83
F-value for 'Treatment'		**	ns	**

[a] Refers to the percentage of annual bluegrass shoots bearing seedheads.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

Table 4.13. Visual quality of 'Penncross' creeping bentgrass on a putting green as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Treatment	Rate L/g ha^{-1}	Turfgrass quality rating[a], WAT[b] 3	4	5	7	9
Untreated control		8.00	8.00	8.00	8.00	8.00
Xanthomonas campestris[c] alone	9.46 L	8.00	8.00	8.00	8.00	8.00
Trinexapac-ethyl alone	0.43 L	9.00	9.00	9.00	9.00	9.00
Trinexapac-ethyl + *Xanthomonas campestris*	0.43 L 9.46 L	9.00	9.00	9.00	9.00	9.00
Paclobutrazol alone	358.00 g	8.00	9.00	9.00	8.00	8.00
Paclobutrazol + *Xanthomonas campestris*	358.00 g 9.46 L	8.00	9.00	9.00	8.00	8.00
ECSS 1001 alone	3.43 g	8.00	8.00	8.00	8.00	8.00
ECSS 1001 + *Xanthomonas campestris*	3.43 g 9.46 L	8.00	8.00	8.00	8.00	8.00
F-value for 'Treatment'		**	**	**	**	**

[a] Ratings are based on a 1 to 9 scale, where 1 = dead and 9 = healthy.
[b] WAT = weeks after first application.
[c] A liquid suspension of *Xanthomonas campestris* containing 10^9 cfu ml^{-1}; the spray volume was 946 L ha^{-1}.

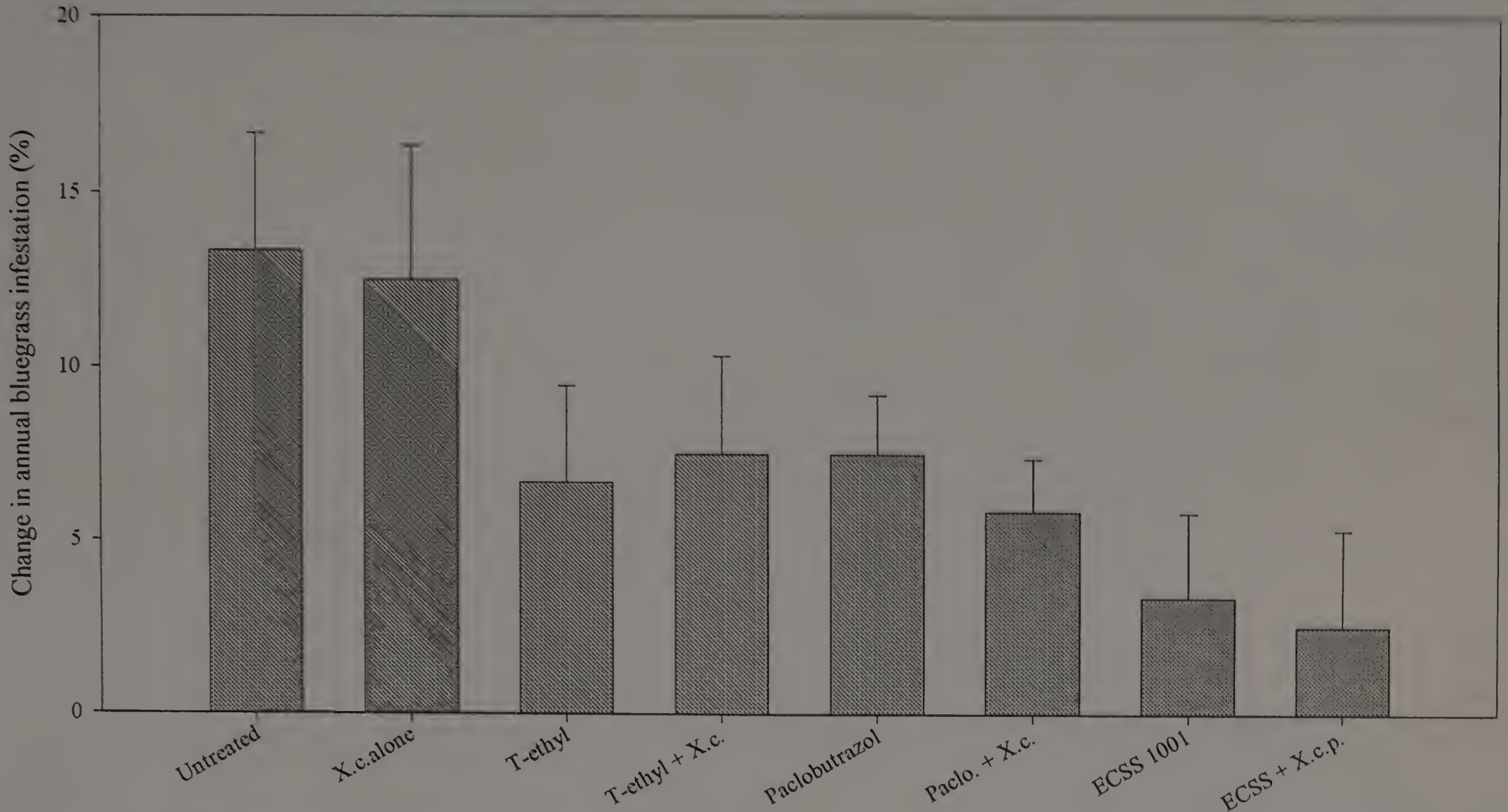

Figure 4.8. Annual bluegrass infestation in a 'Penncross' creeping bentgrass putting green 9 WAT as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

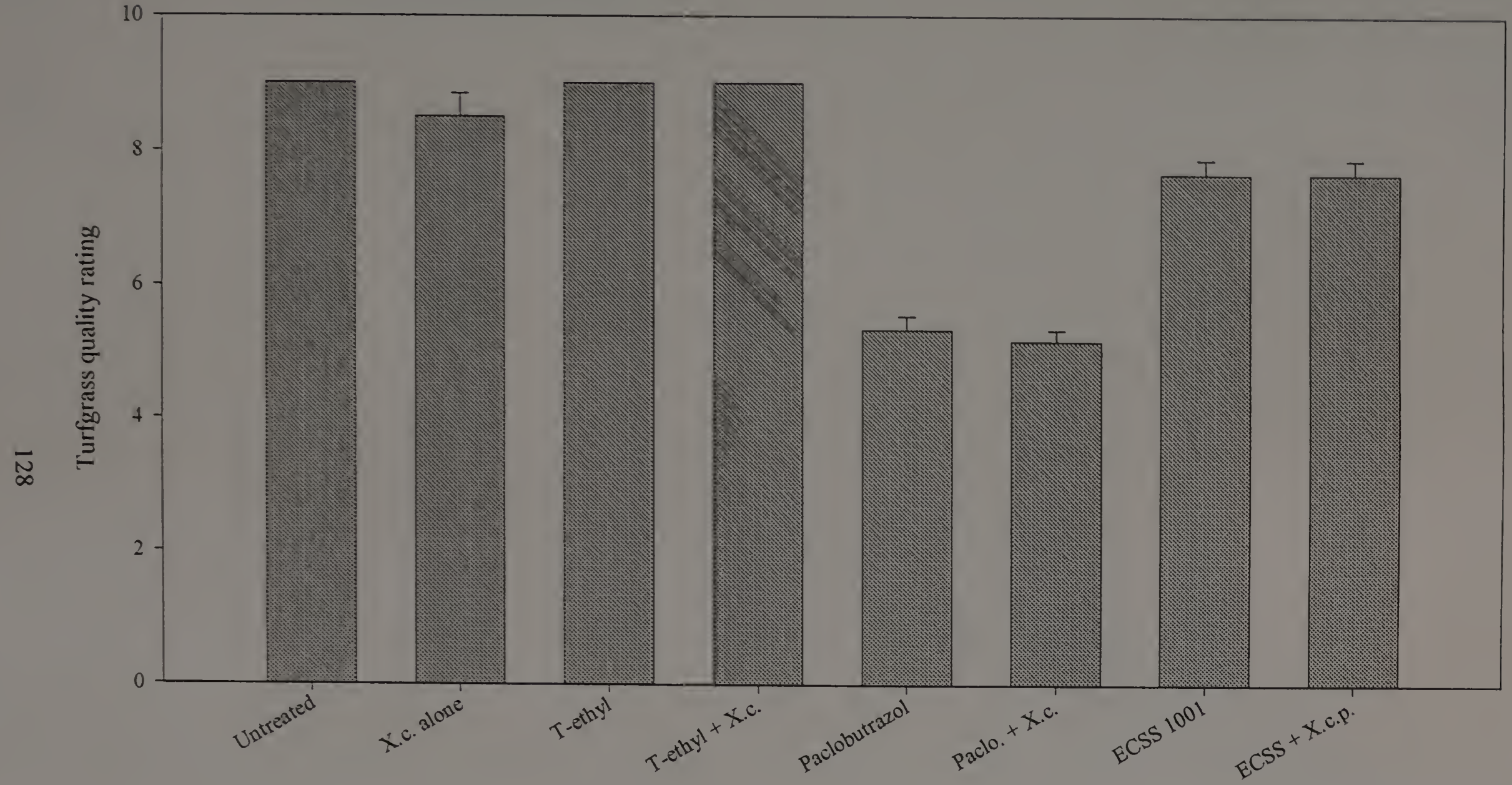

Figure 4.9. Quality of annual bluegrass in a 'Penncross' creeping bentgrass putting green 9 WAT as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Annual bluegrass encroachment (Table A.10), quality (Table A.11), and seedhead production (Table A.12), as well as the quality of creeping bentgrass (Table A.13) were not affected by the combination of *Xanthomonas campestris* and ECSS 1001, as compared to ECSS 1001 alone. Compared to the bacterium alone, the combination of *Xanthomonas campestris* and ECSS 1001 reduced the spreading (Table 4.10) and quality (Table 4.11) of annual bluegrass by 10 and 15% 9 WAT, respectively. Treatment combinations with ECSS 1001 and the bacterium had no effect on annual bluegrass seedhead production, compared to the bacterium alone.

Discussion

Xanthomonas campestris suppressed both biotypes of annual bluegrass by over 90% under controlled conditions, yet caused little or no reductions in annual bluegrass encroachment in field trials, whether applied alone or in combination with plant growth regulators. This is consistent with the findings of Zhou and Neal (1995) who suggested that failure of the bacterium to control annual bluegrass in the field could be attributed to climatic conditions unsuitable for the bacterium in the northeastern U.S.

The annual biotype of annual bluegrass was suppressed by 99% 3 WAT by the bacterium alone and the suppression of the annual biotype was not influenced by trinexapac-ethyl, paclobutrazol, or ECSS 1001, under controlled conditions. Suppression of the perennial biotype of annual bluegrass with *Xanthomonas campestris* alone was 66% 3 WAT, and was increased 27 and 32% 3 WAT by ECSS 1001 and paclobutrazol, respectively. By 4 WAT, 90% suppression of annual bluegrass was achieved in all

treatments with the bacterium, except the bacterium and trinexapac-ethyl. Suppression of the perennial biotype of annual bluegrass, therefore, was accelerated by 1 WAT by using paclobutrazol and ECSS 1001.

ECSS 1001 is an experimental plant growth regulator. In amended media experiments (Chapter II), ECSS 1001 was non-toxic to *Xanthomonas campestris* at concentrations up to 10,000 ppm. Colonies growing on agar containing concentrations of ECSS 1001 higher than 10 ppm grew more rapidly than colonies growing on unamended King's Media B. The concentration of ECSS 1001 within annual bluegrass plants was not determined. It is possible that ECSS 1001 accelerated suppression of the perennial biotype of annual bluegrass by acting as a nutritional cofactor for the bacterium, causing rapid multiplication of the bacterium in the plant.

Trinexapac-ethyl and the bacterium suppressed the perennial biotype of annual bluegrass by 50% 7 WAT. The fact that reduced activity of *Xanthomonas campestris* occurred only within the perennial biotype supports the conclusion of Imaizumi and Fujimori (1997) that perennial biotypes are less susceptible to the bacterium. It may be possible that adequate populations of the bacterium are not established in the presence of trinexapac-ethyl. Furthermore, trinexapac-ethyl is an inhibitor of gibberellic acid biosynthesis. Since gibberellic acid is involved in stem elongation, the rate of vertical growth is slowed by trinexapac-ethyl. It is possible that reduced growth of annual bluegrass caused by trinexapac-ethyl reduces the number and/or severity of mowing wounds, which plays an important role in suppression of annual bluegrass with *Xanthomonas campestris* (Imaizumi et al., 1998).

Paclobutrazol, another inhibitor of gibberellic acid biosynthesis, accelerated suppression of the perennial biotype of annual bluegrass under controlled conditions. One possible explanation for the differential effects of trinexapac-ethyl and paclobutrazol is the difference in formulation. Trinexapac-ethyl was foliar applied, as an emulsified concentrate. Clipping fresh weight of the perennial biotype of annual bluegrass was reduced by trinexapac-ethyl by 74% 3 days after treatment (DAT) and 83% 1 WAT. Paclobutrazol was applied as a granular with a nitrogen base, therefore it had to dissolve and be absorbed by annual bluegrass roots before causing a response. Paclobutrazol reduced clipping fresh weight of the perennial biotype of annual bluegrass by 18% 3 DAT, 57% 1 WAT, and 83% 2 WAT. It may be possible that more numerous and severe wounds were created by mowing in the paclobutrazol-treated plants in the early weeks of bacterial applications due to the delayed effects of the growth regulator. This may have led to the accelerated and more effective suppression of the perennial biotype of annual bluegrass by the bacterium and paclobutrazol, as compared to the bacterium and trinexapac-ethyl. Inhibition of gibberellic acid biosynthesis may have weakened the perennial biotype of annual bluegrass sufficiently to accelerate suppression by *Xanthomonas campestris*, but in the case of trinexapac-ethyl, rapid growth retardation may have prevented sufficient wounding for infection by the bacterium.

In field trials, trinexapac-ethyl, paclobutrazol, and ECSS 1001 were not effective in reducing annual bluegrass populations in a creeping bentgrass putting green, either alone or in combination with *Xanthomonas campestris*. Trinexapac-ethyl and paclobutrazol, applied alone, and in combination with *Xanthomonas campestris* increased

the production of annual bluegrass seedheads by over 20% 7 WAT. For trinexapac-ethyl and paclobutrazol, one possible explanation may be that elongation of seedhead stalks is inhibited by the absence of gibberellic acid and therefore seedheads accumulate below the height of mowing. Quality of annual bluegrass was also reduced by 40% from 5 WAT to 9 WAT.

The quality of 'Penncross' creeping bentgrass was not affected by *Xanthomonas campestris*, trinexapac-ethyl, paclobutrazol, or ECSS 1001, nor by any other treatment combination.

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SUMMARY

Annual bluegrass (*Poa annua* L.) is one of the few weeds able to compete in putting greens, where its presence reduces the aesthetic quality and can influence ball roll on the putting surface. The perennial biotype [*Poa annua* var. *reptans* (Hauskins) Timm], is the dominant biotype found in putting greens in the northern U.S. Selective herbicides are not available for the control of annual bluegrass in putting greens, so golf course superintendents must use an integrated approach for annual bluegrass management. This approach includes cultural, chemical, and recently, biological means of suppressing annual bluegrass.

Xanthomonas campestris pv. *poannua* has potential as a biological control agent for the selective, postemergence control of annual bluegrass in putting greens. The bacterium enters the plant through the mowing wound, and once inside multiplies rapidly, causing xylem occlusion. Research currently is under way to learn more about the interaction between *Xanthomonas campestris* and annual bluegrass, and how that relationship can be influenced to improve suppression of annual bluegrass.

Growth room and field experiments were conducted to determine the effects of various surfactants and plant growth regulators on the activity of *Xanthomonas campestris* in the suppression of annual and perennial biotypes of annual bluegrass. The effects of surfactants, plant growth regulators, and *Xanthomonas campestris* on creeping bentgrass (*Agrostis palustris* Huds.) growth were also examined. Laboratory experiments were conducted to determine the toxicity of various surfactants, growth regulators, and pesticides, to *Xanthomonas campestris*.

Agri-dex was the only surfactant not harmful to the bacterium at recommended rates. Only bifenthrin, among the plant growth regulators and pesticides tested, was toxic to *Xanthomonas campestris* at recommended rates.

Xanthomonas campestris suppressed both annual and perennial biotypes of annual bluegrass by up to 100% in the growth room, yet did not suppress annual bluegrass in field trials when applied alone or in combination with surfactants or plant growth regulators. Agri-dex, at 0.5% (V/V) accelerated infection of the annual biotype of annual bluegrass by 1 WAT, but did not affect the perennial biotype. Silwet L-77, X-77, and high rates of Break-thru [0.6 and 0.9% (V/V)] reduced the activity of *Xanthomonas campestris* by 23, 35, and 87%, respectively, and Break-thru at 0.9% (V/V) injured creeping bentgrass.

Paclobutrazol and ECSS 1001 did not affect suppression of the annual biotype of annual bluegrass by *Xanthomonas campestris*, but accelerated suppression of the perennial biotype by 1 WAT. Combining trinexapac-ethyl with the bacterium reduced its suppression of the perennial biotype of annual bluegrass by 50% 7 WAT. Trinexapac-ethyl and paclobutrazol both increased seedhead production by over 20% 4 WAT in field trials.

Interactions of *Xanthomonas campestris*, annual bluegrass, and other elements of putting green environments must be further examined with regard to bacterial survival and growth, as well as suppression of annual bluegrass. From the results of our amended media studies, it is clear that the bacterium tolerates exposure to low levels of the materials used therein. Further studies should include more diverse materials, and determine the nature of contact between these materials and *Xanthomonas campestris.* Also, the effects of ECSS 1001 on the bacterium should be more closely examined. In general, surfactants had no additive effect on annual bluegrass

suppression by *Xanthomonas campestris*. Therefore further studies should be conducted to determine the relationship of lower surfactant rates and bacterial activity. Timing (relative to bacterial applications) and formulation of plant growth regulators in relation to biological control activity should be considered for future research. The differential results between field and growth room experiments are our main concern. Future research must focus to identify the causes of this type of response in biological control agents.

APPENDIX: ANOVA TABLES

Table A.1. Mean squares from ANOVA for suppression of an annual biotype of annual bluegrass with *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various plant growth regulators.

Source of variation	df	3 WAT[a]	4 WAT	7 WAT
Corrected total	31			
Block	3	213.38	281.08	194.53
Treatment	7	8,026.91**	9,342.64**	9,460.60**
Untreated vs. *X. c. p.*	1	16,200.00**	19,900.13**	17,112.50**
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	312.50	144.50	12.50
X. c. p. vs. paclobutrazol + *X. c. p.*	1	66.13	78.13	3.13
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	28.13	45.13	50.00
Trinexapac-ethyl vs. trinexapac-ethyl + *X. c. p.*	1	12,012.50**	16,653.13**	18,050.00**
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	18,336.13**	17,484.50**	17,578.13**
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	8,778.13**	10,512.50**	12,800.00**
Treatment x block	21	269.45	276.58	160.01

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.2. Mean squares from ANOVA for suppression of a perennial biotype of annual bluegrass with *Xanthomonas campestris* pv. *poannua* under controlled conditions as influenced by various plant growth regulators.

Source of variation	df	3 WAT[a]	4 WAT	7 WAT
Corrected total	31			
Block	3	30.21	333.86	284.11
Treatment	7	7,517.98**	8,923.82**	9,272.21**
Untreated vs. *X. c. p.*	1	8,778.13**	18,432.00**	19,012.50**
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	1,653.13	4,512.50**	5,000.00**
X. c. p. vs. paclobutrazol + *X. c. p.*	1	2,048.00*	3.13	0.00
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	1,458.00	24.50	3.13
Trinexapac-ethyl vs. trinexapac-ethyl + *X. c. p.*	1	2,812.50*	4,704.50**	5,000.00**
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	19,306.13**	18,915.13**	20,000.00**
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	16,928.00**	17,112.50**	16,653.13**
Treatment x block	21	389.59	377.29	453.16

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.3. Mean squares from ANOVA for clipping fresh weight of an annual biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	0 WAT[a]	1 WAT	2 WAT	3 WAT	4 WAT
Corrected total	27					
Block	3	2,732.57**	3,328.90**	654.06*	1,939.73	2,046.66
Treatment	6	2,312.31**	2,325.44**	4,302.11**	7,680.63**	12,332.63**
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	5,128.82**	5,777.05**	700.50	263.93	294.52
X. c. p. vs. paclobutrazol + *X. c. p.*	1	22.88	5,451.77**	4,359.45**	394.66	0.07
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	18.48	16.73	151.12	0.28	6.37
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	61.49	31.48	0.90	8,302.45**	18,117.51**
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	43.43	115.14	594.78	939.83	628.17
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	22.82	331.02	8,634.59**	20,230.66**	32,806.41**
Treatment x block	18	155.64	287.85	172.77	748.14	1162.58

[a] WAT = weeks after first application.

* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.4. Mean squares from ANOVA for clipping fresh weight of a perennial biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	0 WAT[a]	1 WAT	2 WAT	3 WAT	4 WAT
Corrected total	27					
Block	3	5,531.77**	883.32	339.75	416.90	3,432.48
Treatment	6	8,272.87**	2,297.83**	3,933.79**	4,779.04**	8,527.75**
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	10,588.04**	4,458.04**	10.44	350.07	208.79
X. c. p. vs. paclobutrazol + *X. c. p.*	1	987.12	2,087.23*	1,003.52	631.72	225.04
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	3880.81	165.62	0.49	84.70	144.42
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	2.52	40.95	684.87	5.22	3,889.62
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	71.94	750.59	303.81	729.81	1,624.79
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	349.14	0.58	10,372.32**	17,630.66**	26,242.26**
Treatment x block	18	960.85	363.73	392.83	379.98	1,084.03

[a] WAT = weeks after first application.

* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.5. Mean squares from ANOVA for clipping fresh weight of 'G2' creeping bentgrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	0 WAT[a]	1 WAT	2 WAT	3 WAT	4 WAT
Corrected total	27					
Block	3	2,508.24*	934.99	465.21	2,009.57*	9,914.71*
Treatment	6	2,244.99**	5,086.67**	5,474.44**	10,822.29**	10,549.69*
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	6,583.78**	15,885.64**	9,571.75**	27.23	1,052.72
X. c. p. vs. paclobutrazol + *X. c. p.*	1	23.94	12,476.47**	11,460.98**	20,209.55**	25,694.18**
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	27.49	1,020.39	23.77	480.81	160.83
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	394.81	92.82	14.36	155.85	5,215.78
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	152.34	4.88	42.32	454.66	333.47
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	190.13	147.15	80.71	2,701.13*	1,214.01
Treatment x block	18	502.81	403.62	295.81	340.05	1,501.27

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.6. Mean squares from ANOVA for quality of an annual biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	0 WAT[a]	1 WAT	2 WAT	3 WAT	4 WAT
Corrected total	31					
Block	3	0.00	0.31	1.88	1.95	1.98
Treatment	7	0.00	0.31	10.91**	48.78**	56.21**
Untreated vs. *X. c. p.*	1	0.00	0.13	2.00	98.00**	120.13**
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	0.00	0.13	2.00	0.50	1.13
X. c. p. vs. paclobutrazol + *X. c. p.*	1	0.00	0.13	15.13**	0.00	1.13
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	0.00	0.13	18.00**	0.00	0.13
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	0.00	0.00	8.00*	84.50**	98.00**
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	0.00	0.00	28.13**	98.00**	98.00**
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	0.00	0.00	21.13**	55.13**	72.00**
Treatment x block	21	0.00	0.31	1.14	1.61	1.39

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.7. Mean squares from ANOVA for quality of a perennial biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	0 WAT[a]	1 WAT	2 WAT	3 WAT	4 WAT
Corrected total	31					
Block	3	0.21	0.61	1.00	0.86	1.21
Treatment	7	0.41	0.74	5.14	32.60**	43.63**
Untreated vs. *X. c. p.*	1	0.13	0.13	8.00	78.13**	112.50**
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	1.13	2.00	1.13	15.13*	12.50*
X. c. p. vs. paclobutrazol + *X. c. p.*	1	0.00	0.00	2.00	2.00	15.13**
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	1.13	0.13	0.50	1.13	0.00
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	1.13	2.00	0.13	0.50	0.00
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	0.13	1.13	8.00	72.00**	84.50**
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	0.13	0.13	3.13	72.00**	91.13**
Treatment x block	21	0.45	2.45	2.31	2.51	1.66

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.8. Mean squares from ANOVA for quality of 'G2' creeping bentgrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	0 WAT[a]	1 WAT	2 WAT	3 WAT	4 WAT
Corrected total	31					
Block	3	0.00	0.00	0.08	0.00	0.00
Treatment	7	0.00	0.00	1.21**	1.14**	0.00
Untreated vs. *X. c. p.*	1	0.00	0.00	0.00	0.00	0.00
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	0.00	0.00	1.13**	2.00**	0.00
X. c. p. vs. paclobutrazol + *X. c. p.*	1	0.00	0.00	2.00*	2.00**	0.00
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	0.00	0.00	0.00	0.00	0.00
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	0.00	0.00	0.50**	0.00	0.00
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	0.00	0.00	0.00	0.00	0.00
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	0.00	0.00	0.00	0.00	0.00
Treatment x block	21	0.00	0.00	0.06	0.00	0.00

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.9. Mean squares from ANOVA for seedhead production by a perennial biotype of annual bluegrass under controlled conditions as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	0 WAT[a]	1 WAT	2 WAT	3 WAT	4 WAT
Corrected total	31					
Block	3	425.00	719.20	571.88*	40.36	4.95
Treatment	7	692.86	949.67	231.70	111.50	2.57
Untreated vs. *X. c. p.*	1	153.13	112.50	153.13	12.50	3.13
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	112.50	153.13	200.00	0.00	0.00
X. c. p. vs. paclobutrazol + *X. c. p.*	1	612.50	450.00	0.00	50.00	12.50
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	200.00	12.50	153.13	3.13	3.13
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	1,250.00	1,352.00	528.13	153.13	3.13
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	1,250.00	903.13	153.13	50.00	3.13
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	378.13	312.50	3.13	450.00	0.00
Treatment x block	21	645.83	853.96	148.07	108.82	4.95

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.10. Mean squares from ANOVA for annual bluegrass encroachment in a 'Penncross' creeping bentgrass putting green as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df	3 WAT[a]	4 WAT	5 WAT	7 WAT	9 WAT
Corrected total	47					
Block	5	333.33**	1,963.44**	667.50	388.44**	166.77**
Treatment	7	79.46**	2,885.64**	1506.85	91.00**	89.81*
Untreated vs. *X. c. p.*	1	2.083	168.75	8.33	2.08	2.08
X. c. p. vs. trinexapac-ethyl + *X. c. p.*	1	33.33	8,268.75**	1,302.08	102.08**	75.00
X. c. p. vs. paclobutrazol + *X. c. p.*	1	18.75	3,675.00**	1,752.08	52.08**	133.33**
X. c. p. vs. ECSS 1001 + *X. c. p.*	1	252.08**	3,852.08**	133.33	352.08**	300.00**
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*	1	18.75	168.75	300.00	0.00	2.08
Paclobutrazol vs. paclobutrazol + *X. c. p.*	1	8.33	133.33	102.08	2.08	8.33
ECSS 1001 vs, ECSS 1001 + *X. c. p.*	1	8.33	102.08	52.08	2.08	2.08
Treatment x block	35	16.43	395.82	587.02	10.10	28.20

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.11. Mean squares from ANOVA for quality of annual bluegrass in a 'Penncross' creeping bentgrass putting green as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df		3 WAT[a]	4 WAT	5 WAT	7 WAT	9 WAT
Corrected total	47						
Block	5		0.00	0.20	0.54	1.35**	0.38
Treatment	7		1.28**	2.89**	22.69**	9.81**	15.19**
Untreated vs. *X. c. p.*		1	0.00	0.00	0.00	4.08**	0.75
X. c. p. vs. trinexapac-ethyl + *X. c. p.*		1	3.00**	0.00	0.00	0.75	0.75
X. c. p. vs. paclobutrazol + *X. c. p.*		1	0.00	6.75**	40.33**	14.08**	33.33**
X. c. p. vs. ECSS 1001 + *X. c. p.*		1	0.00	0.00	1.33	0.83	2.08**
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*		1	0.00	0.00	0.00	0.83	0.00
Paclobutrazol vs. paclobutrazol + *X. c. p.*		1	0.00	0.00	5.33*	0.83	0.83
ECSS 1001 vs, ECSS 1001 + *X. c. p.*		1	0.00	0.00	0.75	0.83	0.00
Treatment x block	35		0.00	0.11	0.94	0.25	0.18

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.12. Mean squares from ANOVA for annual bluegrass seedhead production in a 'Penncross' creeping bentgrass putting green as influenced by various treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df		3 WAT[a]	5 WAT	9 WAT
Corrected total	47				
Block	5		1,767.08**	1,494.27**	860.52**
Treatment	7		284.52**	288.62	1,158.26**
Untreated vs. *X. c. p.*		1	2.08	133.33	8.33
X. c. p. vs. trinexapac-ethyl + *X. c. p.*		1	408.33**	18.75	2,002.08**
X. c. p. vs. paclobutrazol + *X. c. p.*		1	675.00**	533.33	3,168.75**
X. c. p. vs. ECSS 1001 + *X. c. p.*		1	8.33	352.08	208.33
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*		1	8.33	52.08	18.75
Paclobutrazol vs. paclobutrazol + *X. c. p.*		1	18.75	2.08	2.08
ECSS 1001 vs, ECSS 1001 + *X. c. p.*		1	8.33	52.08	2.08
Treatment x block	35		989.58	383.79	182.90

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

Table A.13. Mean squares from ANOVA for quality of 'Penncross' creeping bentgrass in a putting green as influenced by various Treatments of *Xanthomonas campestris* pv. *poannua* and plant growth regulators.

Source of variation	df		3 WAT[a]	4 WAT	5 WAT	7 WAT	9 WAT
Corrected total	47						
Block	5		0.00	0.00	0.00	0.00	0.00
Treatment	7		1.29**	1.71**	1.71**	1.29**	1.29**
Untreated vs. *X. c. p.*		1	0.00	0.00	0.00	0.00	0.00
X. c. p. vs. trinexapac-ethyl + *X. c. p.*		1	3.00**	3.00**	3.00**	3.00**	3.00**
X. c. p. vs. paclobutrazol + *X. c. p.*		1	0.00	3.00**	3.00**	0.00	0.00
X. c. p. vs. ECSS 1001 + *X. c. p.*		1	0.00	0.00	0.00	0.00	0.00
Trinexapac-ethyl vs. trinex.-ethyl + *X. c. p.*		1	0.00	0.00	0.00	0.00	0.00
Paclobutrazol vs. paclobutrazol + *X. c. p.*		1	0.00	0.00	0.00	0.00	0.00
ECSS 1001 vs, ECSS 1001 + *X. c. p.*		1	0.00	0.00	0.00	0.00	0.00
Treatment x block	35		0.00	0.00	0.00	0.00	0.00

[a] WAT = weeks after first application.
* and ** Significant at 0.05 and 0.01 levels, respectively.

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