THE ROLE OF 7-OXABICYCLO [2.2.1]
HEPTANE-2, 3-DICARBOXYLIC ACID
(ENDOTHALL)
IN ANNUAL BLUEGRASS (POA ANNUA L.)
CONTROL IN TURF

Thesis for the Degree of Ph. D. MICHIGAN STATE UNIVERSITY Alfred J. Turgeon 1971



This is to certify that the

thesis entitled

THE ROLE OF 7-OXABICYCLO[2.2.1]

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(ENDOTHALL)

IN ANNUAL BLUEGRASS (POA ANNUA L.) CONTROL IN TURF

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ABSTRACT

THE ROLE OF 7-OXABICYCLO[2.2.1]HEPTANE-2,3-DICARBOXYLIC ACID (ENDOTHALL) IN ANNUAL BLUEGRASS (POA ANNUA L.) CONTROL IN TURF

Ву

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Experiments were conducted to evaluate the potential of 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid (endothall) for controlling Poa annua L. infestations in Poa pratensis L. and Agrostis palustris Huds. turfs. The absorption, translocation and metabolism of endothall and the effects of endothall on photosynthesis, respiration and transpiration were studied to determine whether they contributed to selectivity among these 3 grasses.

Foliar applications of endothall to single-plant sand cultures of the 3 turfgrass species produced a selective growth suppression of <u>Poa</u> annua at certain rates and frequencies of application. Field spraying of the herbicide produced variable results which were highly dependent upon the season of application.

Root applications to plants in sand culture resulted in a selective kill of <u>Poa annua</u> within a certain concentration range of the herbicide. A granular formulation of endothall provided selective control of <u>Poa annua</u> in some field and greenhouse studies. Variables affecting this response included: watering frequency prior to application, the nature of the underlying soil, and plant variability within the <u>Poa</u> annua species.

The selectivity of endothall in turf was attributed, in part, to differential absorption and metabolism of the herbicide from root applications and the greater sensitivity of the physiological systems of Poa annua to endothall.

Following foliar sprays of endothall, a reduction of photosynthesis and an increase in respiration were observed in <u>Poa pratensis</u> and <u>Agrostis palustris</u>. These effects disappeared within 48 hr. Conversely, the photosynthetic activity of <u>Poa annua</u> was considerably below normal 48 hr after treatment. Root applications resulted in a continuous decrease of photosynthesis in <u>Poa annua</u>, with little effect on the other grasses. Transpiration was severely reduced in <u>Poa annua</u>, and to a lesser extent in <u>Poa pratensis</u>, from root applications of endothall. In contrast, foliar sprays caused no significant effect on transpiration water loss by all 3 turfgrass species.

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I. INTRODUCTION

Annual bluegrass (<u>Poa annua</u> L.) is a low-growing plant that provides a dense, vigorous turf under relatively low cutting heights. It survives reasonably well on compacted soils and is well adapted to moist, shaded conditions. Although it is almost never planted intentionally, it frequently comprises the major component of such turfgrass communities as golf course greens, tees and fairways.

Poa annua is generally considered to be a weed. Its profuse seedhead production under a wide range of mowing heights and its sensitivity to climatic extremes make it an undesirable grass for turf use.

Considerable research has been devoted to finding a suitable procedure for controlling Poa annua. A large number and variety of chemicals have been evaluated for their phytotoxic effects on this plant species, and many have been described as offering some promise for its control; yet Poa annua remains a widespread and perplexing problem for the turfgrass manager.

The herbicide 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxy-lic acid (endothall) was introduced over 2 decades ago and has been useful as a defoliant, desiccant, aquatic herbicide and for preemergence weed control in certain crops. Prior to the development of 2-(2,4,5-trichlorophenoxy)propionic acid (silvex) and 3,6-dichloro-o-anisic acid (dicamba), endothall was used for the control of white clover (Trifolium

repens L.) and knotweed (Polygonum aviculare L.) in turf. In addition, endothall was reported as having some selective action on Poa annua.

The objectives of this study were to determine the nature and basis for the selectivity of endothall in turf and its potential and practical role as a control for Poa
annua. Various foliar application rates and root treatments of endothall were used to determine the range of selectivity. Climatic and soil conditions, plant variability, and methods of application were studied to determine their relationship to the phytotoxic effects of this herbicide.

Absorption, translocation and metabolism of endothall by <u>Poa annua</u> and 2 turfgrass species were studied to determine whether they contributed to selectivity. The effects of endothall on photosynthesis, respiration and transpiration were examined to determine how these physiological processes might be involved in selective action.

II. LITERATURE REVIEW

The Plant - Annual Bluegrass (Poa annua L.)

<u>Description</u>: Hubbard (1959) included all known characteristics of the species in the following description:

A loosely to compactly tufted annual or short lived perennial, 3-30 cm. high. Culms erect, spreading, or prostrate, sometimes with a creeping base and rooting at the nodes, smooth. Leaves green, hairless; sheaths compressed, keeled, smooth; ligules thinly membranous, 2-5 mm. long; blades with abruptly pointed or blunt hooded tips, 1-14 cm. long, folded or opening out and 1-5 mm. wide, weak, often crinkled when young, minutely rough only on the margins. Panicles ovate or triangular, open and loose, or somewhat dense, 1-12 cm. long, pale to bright green, reddish or purplish; branches mostly paired or solitary, spreading, smooth, bare and undivided in the lower part; pedicels 0.3-4 mm. long.

Spikelets ovate or oblong, 3-10 mm. long, 3-10 flowered, readily breaking up beneath each lemma at maturity. Glumes persistent, pointed, keeled; lower lanceolate to ovate, 1.5-3 mm. long, 1-nerved; upper elliptic or oblong, 2-4 mm. long, 3-nerved. Lemmas overlapping, semielliptic or oblong and rather blunt in side view, 2.5-4 mm. long, keeled, 5-nerved, membranous and with broad delicate tips and margins, sparsely to densely hairy on the nerves below the middle, or hairless. Paleas slightly shorter than the lemmas, with hairy or hairless keels. Anthers 0.7-1.3 mm. long. Grain enclosed by the lemma and palea. Ch. no. 2n=28.

Origin and Distribution: Tutin (1952) postulated that Poa annua arose from a cross between Poa infirma H.B.K., an annual species, and Poa supina Schrad., a creeping perennial. This is believed to have occurred in Europe during the Quaternary glaciation. Hitchcock (1935) stated that Poa annua is found in a wide variety of habitats from Newfoundland and Labrador to Alaska, south to Florida and

California, and at high altitudes in Tropical America. Its occurrence has also been recorded in Australia, South America, North Africa and North Asia (Gibeault, 1970). Tutin (1952) observed that it is usually limited to areas of human habitation. Hovin (1957) postulated that it was introduced to America by the early Spanish explorers.

Reproduction: The propagation of <u>Poa annua</u> is primarily by seed (Beard, 1970; Harper, 1965; Hawes, 1965; Sprague and Burton, 1937). Renney (1964) observed that a single plant produced over 360 seeds between May and August in western British Columbia. Recent investigations into the perenniality of some biotypes by Gibeault (1970) suggest that vegetative propagation by stolons may also be operative in the survival and spreading of the plant.

Hovin (1958) observed that \underline{Poa} annua is almost always self-pollinating but that by growing the plant in maximum day temperatures of 28.0 ± 1.6 C and 20.0 ± 1.2 C minimum night temperatures, the anthers could be shrivelled and made nonfunctional. Cross-pollination then produced 96% hybrids in the F_1 generation. Koshy (1969) reported that "sexual reproduction in \underline{Poa} annua combines an efficient mechanism of pollen liberation and stigma exsertion which promotes cross-pollination and self-incompatibility." He concluded that self- and cross-pollination are equally probable in terms of the timing of pollination, since pollen liberation does not occur in the closed floret. He also observed that seed formation can proceed following pollination even when the

panicles are removed from the plant on the same day pollination occurs. Hence, the remarkable efficiency of seed formation contributes substantially to the success of Poa annua as a weed.

Youngner (1959) determined that flowering was not governed by day length and only slightly affected by temperature within the range of 10 to 27 C. Consequently, seed production may occur throughout the entire growing season. It has been widely observed, however, that seedheads are most abundant in spring.

Seed Germination: Cockerham and Whitworth (1967) reported that freshly produced <u>Poa</u> annua seed, in New Mexico, were dormant. These required several weeks aging and temperatures below 27 C during the day and nighttime temperatures of 10 to 21 C before germination would occur. Tutin (1957) observed that seed of var. <u>reptans</u>, a perennial biotype, germinated soon after production but that an annual variety usually required a 3-month ripening period before germination would take place.

Germination of <u>Poa</u> <u>annua</u> normally takes place in late summer or early fall, with spring germination occurring in some areas (Harper, 1965). Engel (1967) found that alternating temperatures of 30 C day and 20 C night resulted in higher percentage germination than 30 C day and 10 C night, or constant temperatures of 19 C and 30 C. Also, comparisons of dark-germinated seeds with those receiving 8 hours of light per day revealed significantly higher germination

when the seeds were exposed to light. These results were supported by Neidlinger (1965), who also demonstrated that light was essential for optimum germination.

Growth: Harper (1965) reported that seedling <u>Poa</u> annua plants develop quite rapidly and produce thick stands despite the lack of creeping stems. Furthermore, growth might occur during late fall, winter and early spring as long as the soil remains unfrozen.

Juhren et al. (1957) measured the growth rate of Poa annua under various temperatures, photoperiods and light intensities. They determined that optimum growth occurred at 26 C day temperature and 17 C night temperature with a 16-hr photoperiod and high light intensity. Sprague and Burton (1937) concluded from experiments in New Jersey that continuous light shade was more favorable to Poa annua, in summer, than full sunlight. They attributed this to the cooler temperature and higher humidity associated with the shaded environment. Hawes (1965) observed that the optimum soil temperature for root growth of Poa annua was approximately 16 C. At this temperature the plants had a distinctly horizontal growth as compared to a more vertical development at 32 C. Beard (1970) reported that optimum shoot and root growth occurred at approximately 15 to 21 C and 10 to 15 C, respectively.

Youngner (1959) showed that the growth and survival of Poa annua were highly dependent upon continuous availability of soil moisture. Observations of this type have led to the

common belief that <u>Poa</u> <u>annua</u> is a typically shallow-rooted plant. Sprague and Burton (1937) determined, however, that shallow rooting was a response to compacted soil conditions and that, with a more favorable soil structure, <u>Poa</u> <u>annua</u> produced roots comparable to those of Kentucky bluegrass (<u>Poa</u> <u>pratensis</u> L.) and colonial bentgrass (<u>Agrostis</u> <u>tenuis</u> Sibth.).

Susceptibility to Environmental Stress: Poa annua is inferior to perennial turfgrasses in its hardiness to heat stress (Beard, 1970). Temperatures as low as 40 C might cause a direct high temperature kill of the plant and even lower temperatures may result in injury if the plants are also subjected to a moisture stress (Beard, 1968).

Low temperatures might also be especially injurious to Poa annua (Beard and Olein, 1963). This results from mechanical disruption of the protoplasm by ice crystals during winter and early spring. Ice crystal formation is largely dependent upon the hydration level of the plant tissue. Even if direct low temperature kill does not occur, the roots of Poa annua might be injured sufficiently to limit the water uptake capability of the plant so that rapid transpiration in spring might cause desiccation and death.

Beard (1970) reported that the relative tolerance of

Poa annua to submersion was inferior to bermudagrass (Cynodon dactylon L. Pers.), bentgrass, zoysiagrass (Zoysia

Willd.) and Kentucky bluegrass. Also, it had a much higher
wilting tendency than most other turfgrasses where there

was a deficit or excess of moisture. Drought and wear tolerance were also poor.

Bobrov (1955) found that <u>Poa</u> <u>annua</u> was very susceptible to smog in Los Angeles, California. This might be of considerable significance in urban industrial areas where atmospheric pollution might reach phytotoxic levels. Injury appeared as a light brown band between the tip and midsection of the leaf blades. Microscopic examination revealed chloroplast disintegration, plasmolysis and dehydration of mesophyll cells in the leaf tissue.

Diseases to which <u>Poa annua</u> is susceptible include: anthracnose, <u>Helminthosporium</u> leafspot, <u>Fusarium</u> snow mold (Sprague and Evaul, 1930), <u>Septoria</u> leafspot, <u>Fusarium</u> root and crown rot, dollar spot, brown patch, red thread, <u>Ophiobolus</u> patch, rust and leaf smut (Couch, 1962). There is evidence that some fungicides are less effective on <u>Poa annua</u> than on bentgrass for the same diseases. McCullough (1953) found that <u>Poa annua</u> required much higher rates of mercury fungicides for snow mold control than did bentgrass.

Subspecies Variability: Poa annua is generally considered a short-lived plant, completing its life cycle within one growing season (Harper, 1965). Goss (1965) reported that it may live as a perennial provided no climatic extremes are encountered. McCullough (1953) determined that Poa annua is a biennial in the Alberta region of Canada. Tutin (1957) observed both annual and perennial growth forms in England. Hovin (1957) noted that perennial biotypes form secondary

tillers on the upper nodes of the culm and produce more tillers per plant than the annual forms. Timm (1965) reported that most samples collected in Europe were perennial or biennial. These were procumbant and had a strongly fibrous root system, while the annual types were erect and exhibited a less extensive rooting habit. He proposed the following subspecies designations: annua (var. typica Beck.), for the upright-growing annual types; reptans (var. reptans (var. aquatica Aschers.), for those plants found on ditchbanks and adjacent to bodies of water. Since cross-pollination between the upright annual and the creeping perennial is possible, many intermediate forms probably occur.

Although Hovin (1957) concluded that the most common type of Poa annua in the United States was the upright annual, Gibeault (1970) found a high proportion of perennial types in samples collected in Oregon and Washington. He determined that the presence of annual or perennial subspecies in a turf could be correlated with the watering regime. Areas that were watered infrequently or not at all would have mainly annual types, while the perennial subspecies could be anticipated in more frequently irrigated turfs.

Cultural Control: Weed control in turf is based primarily on a comprehensive program of cultural practices designed to maximize the competitive ability of the desired turfgrass species. Furgeson (1959) proposed that greens should be fertilized when Poa annua is weakest in comparison

to bentgrass, and that irrigation water should be supplied infrequently but thoroughly to favor deep rooting of the desired species. He also recommended periodic alleviation of soil compaction and disease and insect control. (1967) suggested that late summer overseeding could help some bentgrasses gain a head start over Poa annua. Schery (1968) noted that the correct choice of turfgrass varieties is an important competitive control for Poa annua. (1961) suggested withholding spring fertilization until the bentqrass or Kentucky bluegrass has initiated growth. Conversely, late summer or fall fertilizer applications should be made before Poa annua begins to make its vigorous cool weather growth. Sprague and Burton (1937) reported that turf made strongly acid by the use of acid-forming fertilizers did not allow entry of Poa annua because of the low tolerance of this species for high acidity. They cautioned, however, that bentgrass also suffered from high soil acidity and that intentional reduction of pH was unsatisfactory as a means of controlling Poa annua.

Chemical Control: Attempts to control <u>Poa annua</u> with chemicals began over 40 years ago. Sprague and Burton (1937) reported that applications of lead arsenate greatly reduced the abundance of <u>Poa annua</u> in creeping bentgrass turf.

Daniel (1955) reported that calcium arsenate, lead arsenate and sodium arsenite were effective in removing <u>Poa annua</u> from Kentucky bluegrass and colonial bentgrass turfs. He further indicated that arsenic toxicity was inversely related

to the soil phosphorus level. Engel and Aldrich (1960) reported substantial reductions in <u>Poa annua</u> from successive applications of sodium arsenite in combination with 2,4-dichlorophenoxyacetic acid (2,4-D).

Injury to bentgrass turf has been observed following application of arsenic compounds (Daniel, 1955; Cornman, 1964). Engel et al. (1968) reported that the quality of bentgrass fairway turf was often reduced to 50% of normal from calcium arsenate. Also, late summer applications were generally more damaging than mid-spring applications.

Goetze (1956) found that fall applications of 1-butyl-3-(3,4-dichlorophenol)-1-methylurea (neburon) gave excellent control of Poa annua with no sustained injury to the desirable bluegrass turf. Mruk and DeFrance (1957) reported fair to moderate control in athletic field turf with disodium methanearsonate (DSMA), isopropyl m-chlorocarbanilate (chlor-propham) and neburon. Several copper compounds showed promise for the control of Poa annua without objectionable turfgrass injury in tests by DeFrance and Kolett (1959).

Fluorophenoxyacetic acids were reported to induce sterility in Poa annua for 4 to 6 weeks (Anderson and McLane, 1958). Also, 1,2-dihydro-3,6-pyridazinedione (maleic hydrazide) reduced the number of Poa annua seedheads but also seriously reduced the bentgrass content of the turf and allowed a substantial increase in white clover (Engel and Aldrich, 1960). Goss (1964) found that dimethyl tetrachloroterephthalate (DCPA), O-(2,4-dichlorophenyl)O-methyl

isopropylphosphoramidothioate (DMPA), a,a,a,-trifluoro-2,6dinitro-N, N-dipropyl-p-toluidine (trifluralin), O,O-diisopropyl phosphorodithioate S-ester with N-(2-mercaptoethyl) benzenesulfonamide (bensulide) and N,N-dimethyl-2,2-diphenylacetamide (diphenamid) effectively inhibited either germination or subsequent growth and development of Poa annua seedlings. His results indicated that overseeding with desirable grasses should be delayed at least 12 weeks after application of these materials. Gibeault (1967) observed that DCPA, trifluralin, bensulide and N-butyl-N-ethyl-a,a,a,-trifluoro-2,6-dinitro-p-toluidine (benefin) effectively controlled Poa annua that was artificially introduced into sea-marsh turf (Agrostis palustris Huds. and Festuca rubra L.). Cornman et al. (1964) reported injury to bentgrasses with DCPA and DMPA but not bensulide. Furthermore, Poa annua control was reduced when these materials were applied to soils with high phosphorus levels (Juska and Hanson, 1967).

The list of herbicides that have, at times, shown promise for the control of <u>Poa annua</u> is extensive. Continued evaluation of these materials, however, has often produced inconsistent results (Engel <u>et al.</u>, 1968). Further testing of these herbicides in specific use situations is necessary to clarify their respective roles in the control of <u>Poa annua</u>.

The Herbicide - Endothall

Applications to Turf: Nutter et al. (1951) observed a temporary discoloration of turf from endothall applied in

October at the rate of 1.1 kg/ha. Varying the spray volume from 94 to 1870 1/ha (10 to 200 gal/A) did not significantly affect the degree of discoloration. Higher application rates produced correspondingly greater turfgrass injury. and DeFrance (1953) reported temporary but severe turfgrass discoloration from endothall applied in July at 2.2 kg/ha. They also concluded that varying the spray volume from 234 to 1870 1/ha did not influence the degree of discoloration. Jagschitz (1954) observed that creeping bentgrass (Agrostis palustris Huds.) developed greater discoloration than Kentucky bluegrass and that mowing the grasses before application generally increased the degree of discoloration. sumably, this would allow greater penetration of the herbicide into the fresh wounds of the turfgrass leaves. Recently, Turgeon and Meggitt (1971b) reported browning of Kentucky bluegrass from endothall applied at rates of 2.2 and 4.5 kg/ha; however, the turf had completely recovered 4 weeks after application.

Engel and Aldrich (1960) reported profound differences in turfgrass discoloration depending upon the season of application. Severe injury resulted from most fall applications of endothall to bentgrass turf, while only slight injury was observed after spring applications. In addition, they made spring applications of endothall for 4 consecutive years to a bentgrass fairway having a uniform distribution of Poa annua. A series of 2 or 3 treatments, at 0.56 kg/ha, was applied at 2-week intervals each spring. This resulted

in a 37% reduction in <u>Poa</u> annua the first season and a 62% reduction at the close of the fourth season when compared to the untreated check. The corresponding increase in bentgrass was 37% and 55%.

Absorption: Poland (1952) suggested that the highly polar nature of endothall limits its penetration into plant surfaces. Maestri and Currier (1958) noted that maleic hydrazide enhanced penetration of endothall. They attributed this response to the surfactant and/or humectant effect of the maleic hydrazide formulation. Tischler et al. (1951) found that the addition of a surfactant to the spray solution increased the effectiveness of endothall. Stahler (1950) observed a considerable increase in the activity of endothall with the addition of ammonium sulfate.

Translocation: Linder (1951) found that injury from foliar-applied endothall was confined to the sprayed portions of oat (Avena sativa L.) and bean (Phaseolus vulgaris L.) plants. Application of endothall to the soil, however, resulted in complete kill of these plants. He interpreted these observations as evidence of little or no basipetal translocation, although acropetal transport of the herbicide was possible. Maestri and Currier (1958) showed that, at certain concentrations, endothall prevented basipetal movement of ¹⁴C-maleic hydrazide in barley (Hordium vulgare L.). More recently, Maestri (1967) reported essentially no translocation of ¹⁴C-endothall in bean plants, but rapid apoplastic and some symplastic movement in cucumber (Cucumis

sativus L.) plants. Thomas (1966) reported that ¹⁴C-endothall moved from treated leaves in a symplastic pattern in pondweed (<u>Potamogeton nodosus Poir.</u>) and water-weed (<u>Elodea canadensis Gray</u>). Furthermore, Belonsov (1960) observed that radioisotopes of phosphorus and sulfur applied to the leaves of cotton (<u>Gossypium hirsutum L.</u>) plants moved to the ripening capsules at a faster rate when endothall was sprayed on the leaves.

Metabolism: Hiltibran (1962) employed a flax-seed (Linum usitatissimum L.) bioassay to determine endothall residue in aquatic systems. He reported that applications to achieve up to 10 ppm in field water tanks could not be detected after 60 hr. He also observed that the addition of mud and plant debris hastened the disappearance of endothall in aquaria. This suggests that endothall degradation is related to the presence of microorganisms. witz (1966) observed that endothall residues persisted longer on dry than on moist soils. In addition, he reported that successive applications of endothall resulted in a faster degradation of the herbicide in soil. This is in agreement with some established principles of microbial decomposition of herbicides as defined by Kearney and Kaufmann (1969). An initial application of some herbicides stimulates microbial population growth in a pattern illustrated by a sigmoidal curve. Subsequent applications intercepting a point at which the microbial population is large are degraded more quickly, as more microorganisms capable of using the herbicide as a substrate are present.

The incorporation of degradation products of endothall into carbohydrates was reported by Freed et al. (1961).

This was determined in tests with beet (Beta vulgaris L.) and spinach (Spinacia oleracea L.) plants. Montgomery and Freed (1964) have shown that plants, fish and soil microorganisms can completely metabolize endothall. Although the main degradation product in microorganisms was carbon dioxide, the radioactive label was found in various biochemical components in the plants and fish. They suggested that the first point of attack on the endothall molecule was probably the endoxo bridge, with subsequent formation of organic acids containing hydroxy or keto functional groups.

Physiological Effects: A continuous decrease in the photosynthetic activity of young apple (Pyrus malvus L.) plants treated with endothall was reported by Rakitin and Imamaliev (1959b). The chlorophyll content of the leaves also decreased, although higher endothall concentrations lessened the chlorophyll destruction. Maestri (1967) observed grana disintegration and chloroplast shrinkage following endothall treatment.

Hall (1952) observed that respiration of detached cotton leaves decreased for about 2 hours following endothall application and then increased to about twice the rate of the control. Currier (1953) found a marked increase in respiration of Elodea leaves treated with endothall.

Maestri and Currier (1958) reported that endothall increased respiration in root tips and leaf segments of barley. In

apple tree leaves treated with endothall, Rakitin and Imamaliev (1959b) observed increased respiration accompanied by an increase in the activity of oxidative enzymes.

Dunning (1958) concluded that endothall stimulates callose formation in either parenchyma cells or in the sieve elements. Maestri (1967) observed that endothall induced callose formation in beans and cucumber in the time sequence: epidermis, veinlet endings, spongy mesophyll and vein parenchyma, and palisade cells. This sequence generally correlates with the presumed path of endothall in the leaves. Following root applications, endothall promoted callose deposition in sieve tubes in the leaves.

Rakitin and Imamaliev (1959b) reported a considerable increase in the content of monosaccharides and a decrease of disaccharides and starch in applies treated with endothall. Also, there was evidence of a conversion of organic phosphorus to an inorganic form in the endothall-treated plants. The authors further stated that endothall caused an accumulation of inorganic nitrogen in the leaves, followed by a decrease in all forms of nitrogenous substances.

Mann et al. (1965) suggested that protein synthesis might be inhibited by endothall.

Currier and van der Zweep (1956) observed cytoplasmic swelling and streaming in plasmolyzed leaf cells of <u>Elodea</u> treated with endothall. Maestri (1967) also reported accelerated protoplasmic streaming in Elodea and suggested

that this might be due to the higher respiratory rates caused by endothall.

Cell membrane permeability changes are suggested by the observations of Rakitin and Imamaliev (1959a), as the ability of apple leaves to retain water decreased and transpiration increased during the first day following spraying with endothall. Afterwards, this effect reversed—leaf water content increased and transpiration decreased. Maestri (1967) reported other membrane effects due to endothall, including ion leakage, modified tonoplast plasmolysis, water loss and browning of leaf tissue. He also suggested that endothall is probably bound to the membranes by ionic interactions, resulting in a general molecular disorder and a disturbance of cell compartmentation.

III. MATERIALS AND METHODS

Single-plant sand cultures of Kentucky bluegrass (Poa pratensis L. cultivar Merion), creeping bentgrass (Agrostis palustris Huds. cultivar Penncross) and annual bluegrass (Poa annua L.) were used in much of this study. Seeds were germinated in sandy loam soil in small plastic boxes and the resulting plants were transplanted singly into washed, coarse sand. The sand containers were 180-ml (6-oz) styrafoam cups with several holes punched in the base for drain-These, in turn, were placed in 300-ml (10-oz) plastic cups so that the tops of both cups were nearly flush. created a volume between the bases of the two cups which functioned as a well for a nutrient solution (Hoagland and Arnon, 1950) that was added 2 to 3 times per week. An adequate moisture level was maintained in the sand cultures by cutting small slits in either side of the plastic cups approximately halfway between the top and base. The sand cultures were first placed under a mist irrigation system in the greenhouse to enhance survival of the grass seedlings and were then moved to an adjacent bench, where they were watered daily by hand. The plants were generally suitable for experimental use 6 to 8 weeks after transplanting.

Herbicide applications in the greenhouse were done with a carbon dioxide-propelled system utilizing a fixed-position nozzle and a revolving belt for transporting plants under the spray swath. The standard spray volume was 935 1/ha.

Field spraying was done with a carbon dioxide-propelled small plot sprayer developed by Turgeon and Meggitt (1971a). Spray volume was 262 1/ha.

A commercial product, Endothal Turf Herbicide ^R, containing 19.3% disodium endothall (15.5% acid equivalent) was used for all foliar-spraying and root-application studies. An experimental granular formulation of 2.5% disodium endothall on a celatom carrier was used in some greenhouse and field studies.

Plugs, boxes and flats of turf were maintained in the greenhouse and used for some of the studies. The soil used in these containers was a sandy loam, except where indicated otherwise. The turf was clipped 3 times per week with a portable mowing unit. In addition, the plants were watered daily and fertilized weekly with a 28-18-8 soluble fertilizer in sufficient quantity to maintain healthy growth.

Selectivity

Differential Growth Response: Single-plant sand cultures of Poa pratensis, Agrostis palustris and Poa annua were clipped to a uniform height of 4 cm and then sprayed 1, 2 or 3 times, at weekly intervals, with 0, 0.6, 1.1, 2.2, 4.5 or 9.0 kg/ha of endothall. Each treatment was replicated 4 times. Plant height was measured after 31 days by grasping the foliage with the fingers and measuring foliar length from the sand surface to the mean leaf terminals. Root dry weights were also determined after washing and severing the roots from the plants and drying them at 105 C for 16 hr.

Another series of sand cultures of the 3 grasses each received 75 ml of a 0, 10, 100 or 1000 ppm solution of endothall applied to the sand in 4 replications. Care was taken so that none of the herbicide contacted the foliage. The sand cultures were placed in the greenhouse at a temperature of approximately 22 C and were watered daily. In addition, nutrient solution was applied 3 times per week. The plants were checked daily over a 3-week period for phytotoxic effects.

The phytotoxic response of the herbicide concentration between 0 and 100 ppm was then studied to determine the lowest concentration at which selective kill could be achieved. Plants were treated with 75 ml of a 0, 25, 50, 75 or 100 ppm endothall solution and then placed in the greenhouse. Each treatment was replicated 4 times. Phytotoxicity from the herbicide treatments was visually determined after 3 weeks.

Spray Volume and Surfactant Effects: The 3 grass species in sand culture were sprayed with endothall at 2.2 kg/ ha and at spray volumes of 187, 935 and 1870 1/ha. Measurements of plant height were taken at 6, 18 and 25 days following treatment. The plants were clipped to 4 cm after each measurement.

A second series of plants was sprayed with endothall at 2.2 kg/ha with a surfactant (alkylarylpolyoxyethylene glycols, free fatty acids and isopropanol) at 0.5% of the spray solution and without. Spray volume was 935 l/ha. Plant height measurements were made at 9, 21 and 28 days after treatment,

and the plants were clipped to 4 cm after each measurement. Each treatment was replicated 4 times and all sand cultures were kept in the greenhouse.

Temperature Effects: Sand cultures of the 3 grasses were trimmed to a uniform height of 4 cm and sprayed with endothall at the rate of 4.5 kg/ha with a surfactant at 0.5% of the spray solution and without. A total of 6 replications of these treatments plus control plants were placed in growth chambers set at 16, 23 or 32 C. Light intensity averaged 2000 ft-candles and a 12-hr photoperiod was used. The plants were watered daily and nutrient solution was supplied 3 times per week. Plant height measurements were taken after 14 days.

Response due to temperature was also studied following root application of endothall. Herbicide concentrations of 0, 25, 50, 75 and 100 ppm were applied to the roots of the 3 grasses in sand culture under the same temperature and light conditions as in the foliar study. Each treatment was replicated 4 times. Phytotoxicity ratings were taken after 7 days.

Soil Composition Effects: Clean sand was mixed with pure bentonite clay to make soil compositions containing 0, 1 and 10% clay (by weight) in sand. Single plants of Poa annua were transferred from sand culture to these 3 soil compositions in 180-ml styrafoam cups. In addition, some were transplanted to cups containing muck soil for an evaluation of organic matter effects on herbicide efficacy.

Each treatment was replicated 6 times. After a 7-day period of adjustment the plants were treated with 75 ml of 50 and 100 ppm endothall applied to the various soil compositions. The plants were placed in the greenhouse for 2 weeks and then visually rated for injury.

A 2.5% granular formulation of endothall was applied to 10-cm plugs of the 3 grass species growing in sandy loam and muck soils. The 4-month-old grass plugs were clipped 3 times per week with a portable greenhouse mower at cutting heights of 4 cm for <u>Poa pratensis</u> and 2 cm for <u>Agrostis</u> palustris and <u>Poa annua</u>. Herbicide application rates included 0, 4.5, 9, 18 and 36 kg/ha of the active ingredient. All treatment combinations were replicated 3 times. The plugs were kept in the greenhouse and then visually evaluated for injury.

Moisture Effects: A series of wooden boxes measuring 25 cm on each side was filled with a sandy loam soil and seeded with Poa annua in one-half of each box and either Poa pratensis or Agrostis palustris in the other halves. The seeding rate was 1 kg/100 sq m. After approximately 2 months, the boxes were placed under 3 different watering regimes; one-third of the boxes were watered daily, while the other thirds were watered every second or third day for a period of approximately 7 weeks. Approximately 1 cm of water was provided in each application. Granular endothall was then applied at the rate of 18 kg/ha to the boxes within each watering regime. Each treatment combination was

replicated 3 times. Visual injury data were taken 2 weeks later to determine the effects of the watering intervals on the susceptibility of the plants to endothall.

Subspecies Variability: Several morphologically differing biotypes of Poa annua were vegetatively propagated in sand culture to accumulate homogeneous groups of intraspecific biotypes. Plants from 4 of these groups were then selected in which 2 groups exhibited the bushy, upright growth habit (annual types) and 2 groups exhibited a less bushy, more prostrate growth habit (perennial types). These plants were treated with 75 ml of 0, 50 and 100 ppm concentrations of endothall applied to the roots. Each treatment was replicated 6 times. The plants were stored in the greenhouse for 2 weeks and then examined for phytotoxic effects.

Field Studies: The first test sites were selected on golf course fairways at 2 locations; one was a Poa pratensis (cultivar Merion) turf with approximately 35% Poa annua distributed uniformly across the plot area (site #1), and the other was an Agrostis palustris (cultivar Seaside) turf with a comparable Poa annua infestation (site #2). Both fairways were irrigated 2 to 3 times per week and mowed twice weekly at approximately 2 cm. Seasonal fertilizer applications provided approximately 3 kg N/100 sq m. The underlying soil was a clay loam at test site #1 and a sandy loam at test site #2. Endothall was sprayed on 2.4 x 1.2 m plots in single applications of 2.2 and 4.5 kg/ha, and 1.1 kg/ha applied 3 times at 2-week intervals. Each treatment was

replicated 3 times. The first treatments were made in early September 1969 and the plots were evaluated visually for injury 7 weeks later and estimates of percent species composition on an area basis were made in the springs of 1970 and 1971.

Endothall was also sprayed onto fairway plots of approximately 50% Agrostis palustris (cultivar Seaside) and 50% Poa annua (site #3). The underlying soil was a sandy loam and turfgrass management conditions were comparable to those of test sites #1 and #2. The herbicide was applied at rates of 0.28 and 0.56 kg/ha at 2-week intervals for a total of 7 applications, beginning May 7, 1970. Plots measured 3.6 x 1.2 m and each treatment was replicated 3 times. Turfgrass injury and the percent species composition of the turfs were determined visually prior to each successive application of endothall.

An identical study was initiated at the same time on a Poa pratensis turf containing approximately 50% Poa annua (site #4). In addition, an adjoining series of plots received the same treatments beginning September 1, 1970, and included a total of 5 applications of endothall. These plots were maintained at a mowing height of 4 cm and supplemental watering was provided as needed. The fertilization schedule provided approximately 2 kg N/100 sq m/yr and the underlying soil was a clay loam. The plots were observed throughout the growing season and visual evaluations of turfgrass injury and percent species composition were made in November 1970 and again in May 1971.

Endothall was applied, in the spring of 1971, as a 2.5% granular formulation to Poa pratensis plots in which 10-cm plugs of Poa annua had been previously transplanted. Test sites were selected at 2 locations in which the underlying soils were muck (site #5) and a sandy loam (site #6), respectively. Plots measured 2.4 x 1.2 m and each contained 6 plugs of Poa annua taken from locations with comparable underlying soil media. Treatment rates were 9, 18, 27 and 36 kg/ha of the active ingredient. The plots were mowed regularly at 4 cm and fertilized to provide 2 kg N/100 sq m/yr. In addition, supplemental irrigation was supplied as needed. The plots were evaluated visually for selective kill of Poa annua approximately 2 weeks after application of the herbicide.

Granular endothall was also applied to an Agrostis palustris fairway containing approximately 40% Poa annua (site #7). Treatments included 9 and 18 kg/ha of the active ingredient. Plots measured 3.6 x 1.2 m and each treatment was replicated 3 times. This study was conducted in the spring of 1971 on the same fairway used for test site #2, and turfgrass management conditions were identical. Turfgrass injury and selective kill of Poa annua were visually evaluated 2 weeks after treatment.

Physiological Investigations

Absorption: Single-plant sand cultures of <u>Poa pratensis</u>, <u>Agrostis palustris</u> and <u>Poa annua</u> were treated with a 10-ul drop of ¹⁴C-endothall (0.14 uCi) applied within a

lanolin ring on a mature leaf blade. This was performed in the greenhouse at approximately 22 C. A total of 4 replications of each species was tested. After 4 hours the plants were washed in distilled water to remove all surface radioactivity. This was followed by freeze-drying for 16 hr, weighing, and combustion to carbon dioxide. A volume of 20 ml of an ethanolamine-ethanol solution (1:2) was injected into each combustion flask to trap the \$14CO_2\$ from the radioactive herbicide. An aliquot of 5 ml was taken from the trapping solutions and added to 15 ml of scintillation solution (5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-2-[5-phenyloxazolyl] benzene/l of toluene) for counting. Data is presented as dpm/plant.

Spray retention was studied using individual leaf blades of each of the 3 grass species. A total of 5 mature leaves per plant was submerged in ¹⁴C-endothall solutions containing 5 uCi/l. This was replicated 6 times for each species. After 10 seconds the leaves were removed and washed in beakers containing 30 ml of distilled water. Aliquots of 0.2 ml of the wash solutions were placed in vials with 15 ml of scintillation solution and counted for 10 min. The leaves were taped to paper and photocopied, and the resulting plant images were cut out and weighed. The total weights were compared to the weight-surface area relationship of the paper for determination of the leaf surface areas. Data were then computed as dpm/sq cm of leaf surface.

Root absorption of endothall was determined by pouring 75 ml of a 50 ppm endothall solution containing 0.75 uCi of ¹⁴C-endothall into sand cultures of the 3 grass species. After 4 hr the plants were washed in distilled water and then freeze-dried for 16 hr. Roots were separated from the foliage and each combusted to carbon dioxide for determination of radioactivity. Six replications of each species were used in this test.

Root absorption was further studied by treating plants in sand culture with 75 ml of 50, 100, 200, 500 and 1000 ppm solutions of endothall containing 0.15, 0.3, 0.6, 1.5 and 3.0 uCi of ¹⁴C-endothall, respectively. Also, <u>Poa annua</u> sand cultures were treated with 75 ml of a 50 ppm endothall solution containing 0.15 uCi of the radioactive herbicide. After 4 hr the plants were washed in distilled water and then were combusted and counted, as in the previous absorption experiment. Each treatment was replicated 4 times. Absorption curves were established for the <u>Poa pratensis</u> and <u>Agrostis palustris</u> plants for comparison with the radioactive level in <u>Poa annua</u>. A duplicate series of plants was treated, but without radioactive endothall, for visual comparison of phytotoxic effects at all concentrations of the herbicide.

Translocation: The mobility of endothall in <u>Poa pratensis</u>, <u>Agrostis palustris</u> and <u>Poa annua</u> was studied with both foliar and root applications.

Each of the 3 grasses in sand culture received a 1-ul drop of ¹⁴C-endothall (0.1 uCi) applied within a lanolin ring to a basal leaf. After 4 and 24 hr, 3 treated plants of each species were radioautographed for 2 weeks in accordance with the procedure of Crafts and Yamaguchi (1964).

In addition, sand cultures of the 3 grasses were treated with 75 ml of a 50 ppm endothall solution containing 1.5 uCi of the radioactive herbicide. After 4 hr, 3 treated plants of each species were freeze-dried and radioautographed.

Metabolism: Sand cultures of the 3 grasses received 75 ml of a 50 ppm endothall solution containing 1.5 uCi of the radioactive herbicide. Each plant was placed in a closed glass system within a 22 C growth chamber. Air was forced into each glass system by a small pump and then channelled into an ethanolamine-ethanol (1:2) solution to trap any ¹⁴CO₂ resulting from the metabolism of ¹⁴C-endothall. After 4 hr of exposure to the root-applied herbicide, air flow from the plant chambers was cut off and 5-ml aliquots of the ethanolamine-ethanol solutions were taken and counted. The dpm obtained were divided by the plant dry weights to express the data on a constant weight basis. A total of 6 replications were used for each species.

Another series of studies was conducted employing thin layer chromatography to determine the nature of the metabolites resulting from endothall degradation. Three plants of each species were treated with 75 ml of a 50 ppm endothall solution plus 0.75 uCi of ¹⁴C-endothall. The plants

were washed in distilled water after 24 hr and extracted with acidified acetone in a Waring blender. The extracts were filtered in a Buckner funnel and additional acetone was used to wash the plant residues. The filtrates were reduced to a 10-ml volume in a water bath with a stream of dry air applied to each beaker. The filtrates were then transferred to 25-ml volumetric flasks with acetone and brought to full volume. Aliquots of 500 ul were spotted on silica gel F-254 plates and developed to 15 cm in a solvent system containing 195 ml of chloroform, ethyl acetate and formic acid (5:4:4). In addition, an endothall solution was spotted on each plate for reference. plates were then allowed to dry overnight, after which visualization of the endothall spots was accomplished with an alkaline solution of bromocresol green. On determining the Rf value for endothall in each case, the plates were scraped in strips measuring 1 cm. These were placed in vials containing 15 ml of scintillation solution and counted for 10 min. The dpm obtained were multiplied by 50 to represent the entire extract volume for each sample, then divided by the fresh weights of the plants for graphical representation.

Photosynthesis and Respiration: Single-plant sand cultures of Poa pratensis, Agrostis palustris and Poa annua were analyzed with an infrared carbon dioxide spectrometer for net photosynthesis and respiration. These determinations were made by measuring the amount of carbon dioxide

in air that had passed through a transparent plastic chamber containing the plants. The plastic chamber was positioned inside a growth chamber set at 22 C. Measurements were made with the plants in darkness, for respiration, and in the light for net photosynthesis. Any variation in the carbon dioxide concentration from that of unexposed air (300 ppm) was due to the photosynthetic or respiratory activity of the plants. Net photosynthesis and respiration rates were calculated as ug $\rm CO_2/min$ and then divided by the foliar dry weight values for each test plant.

After an initial analysis in the spectrometer, 4 plants of each species were sprayed with endothall at the rate of 4.5 kg/ha and a second series received a root application of 75 ml of a 50 ppm endothall solution. The treated plants were analyzed for photosynthesis and respiration at 1, 4, 24 and 48 hr.

Transpiration: Sand cultures of the 3 grass species were prepared with an additional plastic outer cup to prevent leakage of water or nutrient solution from the sand medium. The top surfaces of the sand were covered with filter paper and plastic wrap to prevent evaporative water loss. Top covers were partly removed and 6 plants of each species received 75 ml of a 50 ppm endothall solution. A second series was sprayed with endothall at the rate of 4.5 kg/ha. These and a series of untreated plants were placed in a 22 C growth chamber for 3 days. The sand cultures were weighed at 24-hr intervals to determine the water loss from

transpiration. The plants were removed from the sand at the end of the experimental period, dried at 105 C for 16 hr, and weighed. Transpiration rates were calculated as g water loss/g of plant dry wt at 1, 2 and 3 days after treatment.

IV. RESULTS AND DISCUSSION

Selectivity

Differential Growth Response: Three applications of endothall at 0.6 kg/ha, 2 or 3 applications at 1.1 and 2.2 kg/ha and 1 application at 4.5 kg/ha were adequate to substantially reduce the shoot growth of Poa annua without appreciably affecting Poa pratensis or Agrostis palustris (Table 1). These data were in general agreement with the root dry weights (Table 2). A linear correlation between the 2 sets of data revealed coefficient of regression (r) values of 0.96 for Poa annua, 0.82 for Poa pratensis, and 0.70 for Agrostis palustris.

These results suggest that differential growth suppression of Poa annua in some perennial turfgrass communities might be achieved at specific application rates of endothall. Also, the effectiveness of a particular rate is apparently related to the number of successive weekly applications of the herbicide. The 0.6 and 1.1 kg/ha rates appeared to stimulate root growth of Poa pratensis and Agrostis palustris in some cases (Table 2). This response was not observed with Poa annua.

The 10 ppm treatments of the first study on root application produced browning of the lower leaves of <u>Poa annua</u> and no apparent effect on the other grass species; 100 ppm were sufficient to kill the <u>Poa annua</u> plants with no effect on <u>Poa pratensis</u> or <u>Agrostis palustris</u>; 1000 ppm killed all 3 species (Figure 1).

3 foliar Plant height of 3 turfgrass species in 31 days following 1, 2 or applications of endothall at weekly intervals. Table 1.

Endothall				Pla	nt heic	Plant height, cm ^l			
rate, kg/ha	P. II	P. pratensis	is	AI.	A. palustris	cis	БI	P. annua	m l
		2	3	1	2	3	1	2	3
0.0	0.6	0.6	0.6	15.3	15.3	15.3	12.5	12.5	12.5
9.0	& &	8.5	8.8	14.0	14.8	14.0	11.0	10.8	8.5*
1.1	10.8	0.6	8.5	14.8	15.3	13.5	12.5	7.3*	8.3*
2.2	10.0	0.6	8.3	13.8	14.8	13.8	12.0	7.3*	3.5*
4.5	8.6	*0.9	*0.9	13.8	11.3*	*0.9	6.3*	3.8*	D*2
0.6	8.5	6.3*	4.8*	13.8	9.5*	*	5.3*	3.8*	*

l Values are the means of 4 replications; means in column with asterisk are significantly different from the controls at the 5% level by the Tukey's Range Test.

 2 D = Plants appeared dead; an average height of 2.5 cm was used for statistical analysis.

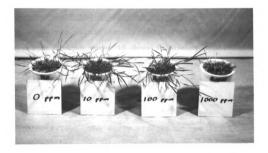
Root dry weights of 3 turfgrass species in 31 days following 1, 2 or 3 foliar applications of endothall at weekly intervals. Table 2.

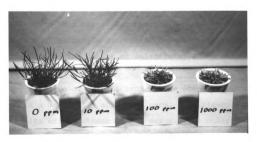
- Prdへ+ball				Ro	Root dry wt, gl	wt, g ¹				
rate, kg/ha	하	pratensis	sis	A.	A. palustris	Tis	ᆈ	P. annua	m1	
		2	3	1	2	3	1	2	3	
0.0	14.1	14.1 14.1	14.1	29.7	29.7 29.7 29.7	29.7	28.4	28.4 28.4 28.4	28.4	
9.0	13.7	15.0	15.0 20.0*	27.5	27.5 51.3* 43.7*	43.7*	28.7	28.7 23.7 22.5	22.5	
1.1	22.5*	17.5 13.7	13.7	35.0	35.0 31.3 27.5	27.5	33.7	15.0* 12.2*	12.2*	
2.2	15.0	11.3 11.3	11.3	27.5	27.5 31.2	31.2	31.3	31.3 11.3*	6.3*	
4.5	15.0	7.5*	8.7*	21.3	8.7*	8.7* 11.2*	5.0*	5.0* 5.0*	5.0*	
0.6	8.7*	7.5*	6.3*	20.0	20.0 16.3* 7.5*	7.5*	5.0*	5.0* 5.0*	5.0*	

l Values are the means of 4 replications; means in column with asterisk are significantly different from the controls at the 5% level by the Tukey's Range Test.

Figure 1. Effects of root-applied endothall on Poa pratensis, Agrostis palustris and Poa annua, from top to bottom, 3 weeks after treatment.







In the second study on root application, the 25 ppm solution produced extensive browning of the lower leaves, while 50 ppm and higher concentrations of endothall caused nearly complete necrosis of Poa annua. The other grass species were apparently unaffected by these treatments (Table 3).

Root application of endothall provided a degree of selectivity that could not be obtained from foliar application. The 50 ppm endothall solution was the minimum concentration at which selective kill was achieved.

Spray Volume and Surfactant Effects: The growth rate of Poa annua was significantly reduced at the 1870 1/ha spray volume for at least 18 days following application.

By 25 days, this effect had essentially disappeared (Table 4). These results indicate that the greater foliar coverage achieved with the highest spray volume may be an important factor associated with herbicidal effectiveness and, in this case, selectivity. The early effects of the 187 1/ha spray volume on Agrostis palustris were manifested as a reduction in shoot growth. This might be interpreted as a concentration effect on this particular species. Hence, the conflicting factors of herbicide concentration and spray volume may, in part, dictate the extent of selectivity among these grass species.

The addition of a surfactant to the endothall solution, applied at a marginally effective rate, significantly enhanced its effectiveness on Poa annua. The other grass

Table 3. Effects of root-applied endothall on 3 turfgrass species 3 weeks after treatment.

Endothall	Tui	rfgrass injury ^l	
conc., ppm	P. pratensis	A. palustris	P. annua
0	1.0 a ²	1.0 a	1.0 a
25	1.0 a	1.0 a	4.0 b
50	1.0 a	1.0 a	8.3 c
75	1.0 a	1.0 a	8.3 c
100	1.0 a	1.0 a	8.7 c

¹ Values are the means of 4 replications; visual injury is expressed using a scale of 1 through 9: 1 = no observable injury and 9 = complete kill of the plant.

² Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

Table 4. Plant height of 3 turfgrass species after treatment with endothall at 2.2 kg/ha applied in 3 spray volumes.

Spray vol.,	Plant height, cm ¹
l/ha	P. pratensis A. palustris P. annua
	6 days after treatment
187	7.5 a ² 7.5 a 10.8 a
9 35	7.5 a 12.5 b 9.5 a
1870	6.8 a 12.0 b 6.8 b
	18 days after treatment
187	15.0 a 16.0 a 14.0 a
9 35	15.0 a 16.0 a 15.0 a
1870	14.0 a 16.0 a 9.6 b
	25 days after treatment
187	8.3 a 10.4 a 10.8 a
9 3 5	9.8 a 12.3 a 10.4 a
1870	10.0 a 11.7 a 8.8 a

 $^{^{\}scriptsize 1}$ Values are the means of 4 replications.

Means in each subcolumn with common letters are not significantly different at the 5% level by the Tukey's Range Test.

species were not so affected (Table 5). The surfactant may provide better foliar coverage and, possibly, greater penetration of the herbicide through the cuticular barrier. The differential effect of the surfactant-endothall combination was not apparent by the fourth week following application.

Temperature Effects: Foliar-applied endothall with or without the surfactant produced no significant effect on Poa pratensis regardless of temperature conditions (Table 6). The growth of Agrostis palustris was suppressed when endothall, in combination with the surfactant, was applied at the 32 C temperature regime. In this case, the surfactant probably aided penetration of the herbicide into the plant and, therefore, enhanced phytotoxicity. In addition, the plants may have been more responsive to the phytotoxic effects of the herbicide-surfactant combination as a direct result of temperature. Poa annua was suppressed at all 3 temperatures following endothall application. The extent of growth suppression was greatest at the intermediate tem-The addition of the surfactant to the herbicide spray enhanced phytotoxicity, causing death of Poa annua at the 23 C and 32 C temperatures. According to Currier and Dybing (1959), warm temperatures and surfactants promote herbicide penetration into the plant through effects on physio-chemical and physiological processes. These include: increased diffusion rate, lowered viscosity, acceleration of photosynthesis, phloem transport, accumulation process, protoplasmic streaming and growth. Possibly, some of these

Table 5. Plant height of 3 turfgrass species after treatment with endothall with or without surfactant.

Endothall	Plant height, cm ¹
rate, kg/ha	P. pratensis A. palustris P. annua
	9 days after treatment
0.0	10.0 a ² 13.0 a 10.8 a
2.2	9.8 a 13.3 a 11.3 a
$2.2 + s^3$	8.8 a 13.8 a 7.5 b
	21 days after treatment
0.0	13.2 a 15.5 a 16.2 a
2.2	13.2 a 14.7 a 16.5 a
2.2 + S	12.8 a 16.2 a 11.7 b
	28 days after treatment
0.0	9.8 a 12.8 a 12.8 a
2.2	10.0 a 13.8 a 12.5 a
2.2 + S	10.8 a 15.0 a 10.8 a

¹ Values are the means of 4 replications.

Means in each subcolumn with common letters are not significantly different at the 5% level by the Tukey's Range Test.

 $^{^{3}}$ S = Surfactant.

Table 6. Effects of foliar-applied endothall after 7 days on the plant height of 3 turfgrass species under 3 temperatures.

Endothall	P	lant height, cm	1
rate, kg/ha	P. pratensis	A. palustris	P. annua
		<u>16 C</u>	
0.0	7.2 a^2	7.7 a	8.3 a
4.5	6.5 a	7.2 a	6.7 b
$4.5 + S^3$	6.5 a	7.0 a	6.8 b
		<u>23 C</u>	
0.0	7.6 a	12.9 a	11.0 a
4.5	7.3 a	11.3 a	6.1 b
4.5 + S	7.4 a	11.4 a	$^{\mathrm{D}}$ c^{4}
		<u>32 C</u>	
0.0	7.0 a	8.0 a	8.3 a
4.5	7.5 a	7.2 a	5.3 b
4.5 + S	7.8 a	4.0 b	Dс

¹ Values are the means of 6 replications.

Means in each subcolumn with common letters are not significantly different at the 5% level by the Tukey's Range Test.

 $^{^{3}}$ S = Surfactant.

⁴ D = Plants appeared dead; an average height of 2.5 cm was used for statistical analysis.

processes were operative in producing the results indicated by the data.

Temperature produced no apparent variability in the response of Poa pratensis or Agrostis palustris to rootapplied endothall (Table 7). Apparently the herbicide concentration range of 0 to 100 ppm was within the tolerance limits of these species over the entire temperature range. This does not mean, however, that the same results would necessarily be obtained under field conditions. field, plants might become conditioned, physiologically, by temperature and other climatic factors so that they are more or less susceptible to injury from a given herbicide. In this study, however, temperature variations subsequent to endothall application did not produce a variable response from the 2 perennial grass species. On the other hand, Poa annua control plants were moderately injured when grown under the 32 C temperature regime. This was attributed to the fact that Poa annua is susceptible to injury from high temperature stress (Beard, 1970). Consequently, the 25 ppm endothall treatment produced generally greater phytotoxicity than at the lower temperatures.

Soil Effects: Soil clay and, particularly, organic matter are significant factors affecting the efficacy of endothall applied directly to the soil. A concentration of 50 ppm of the herbicide was adequate to kill most of the Poa annua plants growing in pure sand; however, the addition of just 1% clay to the sand reduced herbicide efficacy

Table 7. Temperature effects on plant injury measured 7 days after root treatment with endothall.

Endothall	Tur	fgrass injury	
conc., ppm	P. pratensis	A. palustris	P. annua
		<u>16 C</u>	
0	1.0 a ²	1.0 a	1.0 a
25	1.0 a	1.0 a	4.3 b
50	1.0 a	1.0 a	9.0 c
75	1.0 a	1.0 a	8.7 c
100	1.0 a	1.0 a	9.0 c
		23 C	
0	1.0 a	1.0 a	1.0 a
25	1.0 a	1.0 a	4.0 b
50	1.0 a	1.0 a	8.3 c
75	1.0 a	1.0 a	8.3 c
100	1.0 a	1.0 a	8.7 c
		<u>32 C</u>	
0	1.0 a	1.0 a	4.7 a
25	1.0 a	1.0 a	7.3 b
50	1.0 a	1.0 a	8.0 b
75	1.0 a	1.0 a	8.3 b
100	1.0 a	1.0 a	8.7 b

¹ Values are the means of 4 replications and represent visual injury ratings using a scale of 1 through 9: 1 = no observable injury and 9 = complete kill of the plants.

Means in each subcolumn with common letters are not significantly different at the 5% level by the Tukey's Range Test.

by approximately 50% (Table 8). Muck soil was even more effective in reducing the phytotoxic effects of the herbicide. The endothall concentration range for selective kill of Poa annua probably varies widely and is highly dependent upon the nature of the soil composition in which the turf is growing.

The discovery that root-application of endothall provided a greater degree of selectivity between <u>Poa annua</u> and the perennial grass species than did foliar application led to the development of a granular formulation of the herbicide. It was hypothesized that the granular material, when applied to a turf, would essentially bypass the "foliar barrier" and selectively kill <u>Poa annua</u> in a mixed turfgrass sward.

The rate of granular endothall required for selective kill of the <u>Poa annua</u> plugs was dependent upon the nature of the underlying soil medium. Nearly complete kill was achieved with 18 kg/ha of the herbicide when the underlying soil was a sandy loam, while <u>Poa annua</u> growing in muck soil was only moderately injured at that rate (Table 9). Even 36 kg/ha did not produce complete kill of the muck-grown <u>Poa annua</u> plants. The only phytotoxic effect observed with the 2 perennial turfgrass species was some slight injury to <u>Agrostis palustris</u> at 36 kg/ha on a sandy loam soil.

Moisture Effects: Essentially complete kill of Poa annua occurred with 18 kg/ha of granular endothall when the plants were subjected to daily watering. Phytotoxicity

Table 8. Effects of clay and organic matter on the efficacy of root-applied endothall on Poa annua.

	Plant	injury ¹
Soil Composition	Endothall	conc., ppm
	50	100
sand	8.7 a ²	9.0 a
l% clay	5.0 b	7.3 b
10% clay	4.3 b	7.0 b
muck	2.3 c	5.0 c

¹ Values are the means of 6 replications and represent visual injury ratings using a scale of 1 through 9: 1 = no observable injury and 9 = complete kill of the plants.

² Means in column with common letters are not significantly different at the 5% level by Tukey's Range Test.

Table 9. Effects of granular endothall on plugs of 3 turfgrass species growing in sandy loam (SL) and muck (M) soils.

Endothall	Soil	Turfg	rass injury ¹	
rate, kg/ha	type	P. pratensis	A. palustris	P. annua
0.0	SL	1.0 a ²	1.0 a	1.0 a
0.0	М	1.0 a	1.0 a	1.0 a
4.5	SL	1.0 a	1.0 a	1.3 a
4.5	М	1.0 a	1.0 a	1.0 a
9.0	SL	1.0 a	1.0 a	4.3 b
9.0	М	1.0 a	1.0 a	1.0 a
18.0	SL	1.0 a	1.0 a	8.7 d
18.0	М	1.0 a	1.0 a	4.7 b
36.0	SL	1.3 a	2.3 b	9.0 d
36.0	М	1.0 a	1.0 a	7.0 c

Values are the means of 3 replications and represent visual injury ratings using a scale of 1 through 9: 1 = no observable injury and 9 = complete kill of the plants.

Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

decreased as the interval between waterings increased to the 2-day and 3-day schedules (Table 10). No observable injury to Poa pratensis or Agrostis palustris occurred at this rate of the herbicide regardless of the watering regime. The differential effects of granular endothall on the Poa annua plants were probably due to some variability in plant succulence caused by the different watering regimes. In addition, competition for adsorptive sites in the soil between endothall and water, and the resultant differential availability of the herbicide to the plants, may have also been a factor affecting plant response. Field applications of granular endothall probably should be preceded by a period of frequent watering of the turf in order to improve the efficiency in selective Poa annua control.

Subspecies Variability: Single-plant sand cultures of Poa annua frequently had different growth and morphological characteristics. Some were bushy and upright-growing (annual types), while others were characterized by few tillers and the occurrence of prostrate or semiprostrate culms (perennial types). The response variability to endothall in preliminary studies was largely overcome by selecting only those plants exhibiting a bushy, upright growth habit. The responses of the 2 "annual" biotypes of Poa annua to the root treatments with endothall were consistent; both types were completely killed by 50 and 100 ppm concentrations of the herbicide (Figure 2). The 2 "perennial" biotypes, however, were only partially stunted by these treatments and no observable

Table 10. Effects of watering schedule on the susceptibility of the 3 turfgrass species to injury from granular endothall applied at the rate of 18 kg/ha.

Watering	Tur	fgrass injury	
interval, hr.	P. pratensis	A. palustris	P. annua
24	1.0 a ²	1.0 a	8.7 a
48	1.0 a	1.0 a	6.7 b
72	1.0 a	1.0 a	4.7 c

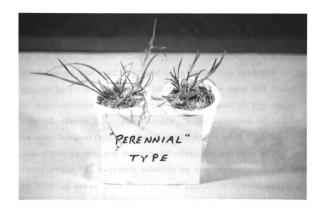
¹ Values are the means of 3 replications and represent visual injury ratings using a scale of 1 through 9: 1 = no observable injury and 9 = complete kill of the plants.

Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

Figure 2. Effects of root-applied endothall on an "annual" type of <u>Poa</u> annua; control plant on the left and treated plant on the right.

Figure 3. Effects of root-applied endothall on a "perennial" type of Poa annua; control plant on the left and treated plant on the right.





browning of the foliage occurred (Figure 3). Hence, the morphological variability among biotypes within the <u>Poa</u> <u>annua</u> species was associated with variable tolerance to endothall.

The degree of <u>Poa annua</u> control obtained from field applications of endothall may vary depending upon the nature of the <u>Poa annua</u> population. The existence of a high proportion of "perennial" biotypes within the <u>Poa annua</u> population may severely limit the effectiveness of control with endothall, while the "annual" component would be expected to succumb fairly rapidly. Gibeault (1970) suggested that the presence of perennial biotypes of <u>Poa annua</u> could account for the variable control results frequently obtained with preemergence herbicides. It is possible that the physiological variability observed in this study may extend to herbicides other than endothall.

Field Studies: Foliar applications of endothall at 1.1 to 4.5 kg/ha in late summer typically resulted in a general browning of the turf followed by a selective recovery of Poa pratensis in about 4 weeks (site #1). The rate and number of applications determined the effectiveness of Poa annua control (Table 11). For example, 3 applications of 1.1 kg/ha were more effective than 1 application of 4.5 kg/ha. Also, the amount and pattern of distribution of the Poa annua infestation had a direct bearing on the capacity of the turf to fill in areas left bare by the herbicide treatments. Large patches of Poa annua (greater than 15 cm in diameter)

Table 11. Effects of foliar-applied endothall on the percent of Poa pratensis and Poa annua in a fairway turf (site #1) 6 months after treatment.

Endothall	Estimated percent cor	mposition of turf ¹
rate, kg/ha	P. pratensis	P. annua
0.0	65 a ²	35 a
2.2	75 b	25 b
4.5	85 c	15 c
1.1×3^3	93 d	7 d

¹ Values are the means of 3 replications.

Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

³ A total of 3 applications was made at 2-week intervals.

remained essentially bare after treatment with endothall, while smaller patches were quickly filled in by Poa praten-sis.

As the residual toxicity of endothall in the soil is very short-lived (Horowitz, 1966), subsequent reinfestation of the turf by <u>Poa</u> <u>annua</u> is largely determined by the cultural practices performed after chemical treatment. Close mowing, frequent watering, high nitrogen fertilization, and traffic encourage the appearance of <u>Poa</u> <u>annua</u> in a turf (Beard, 1970). These same conditions resulted in an extensive reinfestation of <u>Poa</u> <u>annua</u> in the test plots approximately 18 months after treatment.

The Agrostis palustris turf at the test site #2 was severely injured from these treatments and did not fully recover until the following fall. Apparently these rates of endothall are not feasible for use on Agrostis palustris turf.

Lighter application rates of endothall (0.3 to 0.6 kg/ha) did not produce browning of an Agrostis palustris-Poa annua turf during the cool spring weather of 1970; however, the percent species composition of the turfgrass community did not change appreciably during this period (site #3). This was in contrast with the results of Engel and Aldrich (1960), who reported a 37% reduction in Poa annua from 3 spring applications of endothall at 0.6 kg/ha. Continued spraying of these plots in July resulted in extensive browning of the turf that was primarily confined to the Poa annua

component. An evaluation of the treated areas in the spring of 1971, however, revealed complete recovery of the turf, but the percent species composition was essentially unchanged.

An identical study was conducted on a <u>Poa pratensis</u> turf that contained approximately 50% <u>Poa annua</u>. The results from spring applications of endothall were similar to those on the <u>Agrostis palustris</u> plots; no browning of the turf was observed and very little change in the percent species composition occurred (site #4). Continued applications of the herbicide during July did result in turfgrass browning and, in contrast to the <u>Agrostis palustris</u> study, very significant reductions in <u>Poa annua</u> with a proportionate increase in the Poa pratensis component of the plots (Table 12).

These data suggest that <u>Poa pratensis</u> recovers faster from endothall injury during the summer than does <u>Agrostis</u> <u>palustris</u>. If temporary browning of the turf is acceptable, this appears to be an effective procedure in reducing <u>Poa annua</u> infestations in <u>Poa pratensis</u> turf.

Foliar applications of endothall in late summer and early fall produced results similar to those obtained from mid-summer applications. Endothall applications of 0.6 kg/ha, in spring, produced no browning of the turf and had very little effect on the percent species composition of the plots. Under similar climatic conditions in the fall, however, these applications produced extensive browning of the turf and effectively reduced Poa annua infestation. The differential response observed was probably due to a climate-

Table 12. Effects of 7 foliar applications of endothall at 2-week intervals on the percent composition of Poa pratensis and Poa annua in a turf (site #4).

Endothall	Estimated percent	composition of turf ¹
rate, kg/ha	P. pratensis	P. annua
0.0	. 50 a ²	50 a
0.3	68 b	32 b
0.6	83 c	17 c

¹ Values are the means of 3 replications.

Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

induced alteration of the physiological condition of the plants. These observations suggest that considerable importance should be placed on the sequence of climatic conditions prior to endothall application in order to predict the injury potential of this herbicide.

Granular endothall produced highly variable results among the 3 test locations. The muck-grown Poa annua plants (site #5) were little affected by the herbicide, while those growing in the sandy loam soil (site #6) were almost completely killed at the 18 kg/ha rate (Table 13). These data are consistent with those obtained in greenhouse studies in which the efficacy of root-applied endothall was shown to be much lower on Poa annua growing in a muck soil (Table 9). The organic soil apparently adsorbs the herbicide very tightly, making it largely unavailable to the plants. Although the results were much more encouraging on the sandy loam soil, subsequent evaluation of these plots in summer revealed some new Poa annua plants that had apparently germinated in July. This was a common occurrence in nearly all of the field studies and is probably due to the rapid degradation of endothall in the soil.

The third test site (#7) was on an Agrostis palustris fairway that was being maintained under a relatively frequent watering regime. The turf at this location was apparently more sensitive to the granular endothall, as 9 kg/ha were sufficient to provide nearly complete kill of Poa annua component of the plots. This was consistent with results

Table 13. Effects of granular endothall on Poa annua growing on muck (site #5) vs a sandy loam (site #6) soil.

P. annua control ¹		
Muck soil	Sandy loam soil	
1.0 a ²	1.0 a	
1.0 a	5.3 b	
1.3 a	8.3 c	
2.7 b	8.7 c	
2.7 b	8.3 c	
	1.0 a ² 1.0 a 1.3 a 2.7 b	Muck soil Sandy loam soil 1.0 a ² 1.0 a 1.0 a 5.3 b 1.3 a 8.3 c 2.7 b 8.7 c

Values are the means of 3 replications and represent visual injury ratings using a scale of 1 through 9: 1 = no observable injury and 9 = complete kill of the plants.

² Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

obtained in previous studies (Table 10); <u>Poa annua plants</u> that were maintained under a frequent watering schedule were more susceptible to endothall injury than those receiving less frequent applications of water.

The data from these studies indicate that endothall produces a wide variety of responses depending upon the rates and dates of application, the specific formulation of endothall employed, the turfgrass species present, and the type of management. Light foliar applications (0.3 to 0.6 kg/ha) in spring were not effective in substantially reducing the Poa annua component of turfgrass communities. Continuation of these applications in summer or early fall resulted in severe turfgrass discoloration, but significant shifts in the percent species composition only occurred when Poa pratensis was the perennial turfgrass species pre-The employment of higher application rates (1.1 to sent. 4.5 kg/ha) in early fall resulted in very substantial reductions of Poa annua in Poa pratensis turf, especially when several applications were made at 2-week intervals. Again, little benefit resulted from these applications to Agrostis palustris fairways, as this species was either temporarily injured or, at least, unable to spread to areas of dead Poa annua.

Granular endothall offered the greatest promise for selective elimination of <u>Poa annua</u> from <u>Poa pratensis</u> and <u>Agrostis palustris</u> turfs, as little or no discoloration of the perennial grasses occurred from these applications.

The effectiveness of the granular formulation was largely dependent upon the rate of application, the watering frequency, and the nature of the underlying soil. The lowest application rate at which granular endothall selectively killed <u>Poa annua</u> was 9 kg/ha, and this occurred in a frequently watered fairway with a sandy loam soil. Reinfestation by <u>Poa annua</u> would be expected, however, because of the short residual toxicity of endothall in the soil. It is likely that overseeding of treated areas and subsequent application of a suitable preemergence herbicide may aid in establishing desirable perennial turfgrass species.

Physiological Investigations

Absorption: There were no significant differences in the amounts of endothall absorbed by a given area of foliage of the 3 grass species (Table 14); however, Poa annua retained significantly more of the herbicide on its leaf surfaces than did Poa pratensis or Agrostis palustris, allowing for greater total uptake (Table 15). The selective growth suppression of Poa annua (Table 1) may be explained, in part, by this differential absorption. The magnitude of this less than 2-fold difference does not appear adequate to entirely explain the achieved 4-fold level of selectivity based on rates required for injury as shown in Table 1. Other effects are probably operative in determining the basis of selectivity with foliar applications of endothall.

Experiments in which ¹⁴C-endothall was applied to the roots of the 3 grass species showed no significant differences

Table 14. Foliar absorption of endothall by 3 turfgrass species. 1

Species	dpm/plant
P. pratensis	116,320 a ²
A. palustris	121,289 a
P. annua	104,070 a

¹ Values are the means of 4 replications.

Table 15. Retention of ¹⁴C-endothall by the leaves of 3 turfgrass species. ¹

Species	dpm/sq cm
P. pratensis	487 a ²
A. palustris	396 a
P. annua	720 b

¹ Values are the means of 6 replications.

² Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

² Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

in the amount of radioactivity present in the foliage 4 hr after treatment. However, there was an approximate 2-fold increase in the amount of ¹⁴C-label in <u>Poa annua</u> roots compared to <u>Poa pratensis</u> and <u>Agrostis palustris</u> roots (Table 16). Hence, differential absorption of root-applied endothall is probably a factor in determining selective kill of <u>Poa annua</u>. Again, the magnitude of this 2-fold increase appears inadequate for completely explaining the achieved levels of selectivity (Figure 1).

In the subsequent experiment with root absorption, only the 1000 ppm endothall solutions killed the <u>Poa pratensis</u> and <u>Agrostis palustris</u> plants. Some leaf tip burn was evident on plants treated with the 200 and 500 ppm solutions. No phytotoxic effects were observed at the lower concentrations except for <u>Poa annua</u>, which was completely killed when treated with the 50 ppm solution.

The ¹⁴C-endothall concentration in <u>Poa pratensis</u> and <u>Agrostis palustris</u> increased almost linearly with increasing concentrations of the radioactive herbicide in the treatment solutions (Figure 4). The amount of endothall in these 2 grass species after treatment with the 100 ppm solutions was nearly that found in <u>Poa annua</u> treated with a 50 ppm endothall solution. Endothall concentrations in <u>Poa pratensis</u> and <u>Agrostis palustris</u> plants greater than 3 times the phytotoxic concentration of endothall in <u>Poa annua</u> did not cause extensive phytotoxic effects.

<u>Translocation</u>: The 4-hour foliar treatments showed a substantial concentration of the $^{14}\mathrm{C}$ -label in the root

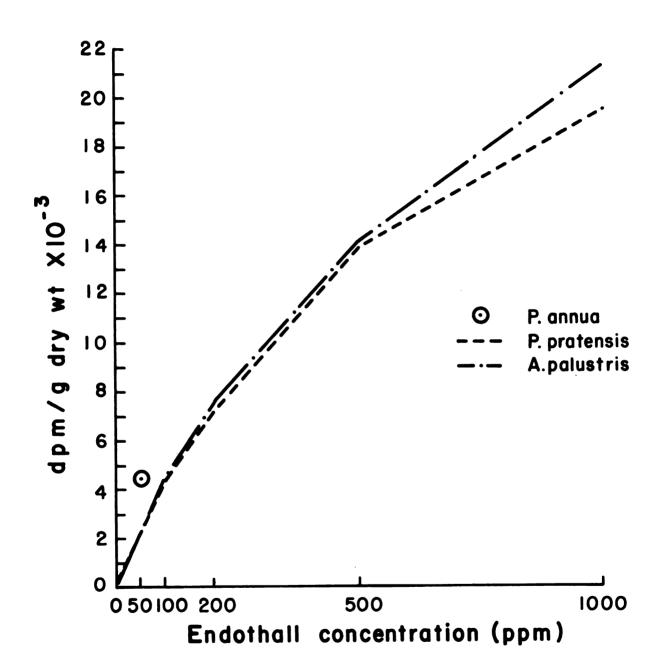
Root absorption of 14C-endothall by 3 turfgrass species.¹ Table 16.

		Radioa	Radioactivity (dpm/g dry wt)	dry wt)
Species		t d	metel	Total . 103
	rorrage	KOOUUS	rocar	14C-endothall applied
P. pratensis	5790 a ²	9229 a	15019 a	9.1 a
A. palustris	4994 a	7613 a	12607 a	7.6 a
P. annua	5254 a	18055 b	23309 b	14.1 b

l Values are the means of 6 replications.

2 Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

Figure 4. Absorption of root-applied 14C-endothall by Poa annua, Poa pratensis and Agrostis palustris.



systems of <u>Poa pratensis</u> (Figure 5) and <u>Poa annua</u> (Figure 9). The 24-hour foliar treatments showed considerable dispersion of the ¹⁴C-label in these grasses (Figures 6 and 10). The <u>Agrostis palustris</u> radioautograms (Figures 7 and 8) showed somewhat slower translocation in comparison to the 2 <u>Poa</u> species.

These treatments represent an application of 8.47 ug of \$14\$C-endothall to each plant. A 1-ul drop of an endothall solution, applied as a 4.5 kg/ha foliar spray with a 935 1/ha spray volume, contains 4.81 ug of the herbicide. Hence, translocation of endothall might be expected to occur from this application under actual field conditions. A smaller spray volume of 187 1/ha would provide over 24 ug of endothall per 1-ul drop. At this concentration, translocation might not occur due to the immediate phytotoxic effects of the herbicide expressed as a foliar burn.

The radioautograms of plants receiving the root treatments showed translocation of the ¹⁴C-label throughout the entire systems of all 3 grass species (Figures 11, 12 and 13). Furthermore, there were no observable differences in the nature of translocation among the 3 species.

The efficiency with which endothall is translocated in these grasses is in marked contrast with the findings of other researchers. Maestri (1967) reported essentially no translocation in bean plants but rapid apoplastic and some symplastic movement in cucumber plants. Linder (1951) found that injury from foliar-applied endothall was confined

Figure 5. Translocation of ¹⁴C-endothall in 4 hours with a foliar application to <u>Poapratensis</u>; whole plant on the left and radioautogram on the right.

Figure 6. Translocation of ¹⁴C-endothall in 24 hours with a foliar application to Poapratensis; whole plant on the left and radioautogram on the right.

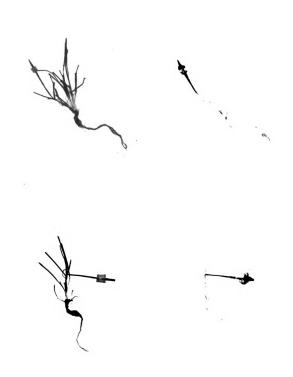


Figure 7. Translocation of ¹⁴C-endothall in 4 hours with a foliar application to Agrostis palustris; whole plant on the left and radioautogram on the right.

Figure 8. Translocation of ¹⁴C-endothall in 24 hours with a foliar application to Agrostis palustris; whole plant on the left and radioautogram on the right.



Figure 9. Translocation of ¹⁴C-endothall in 4 hours with a foliar application to <u>Poa</u> annua; whole plant on the left and radioautogram on the right.

Figure 10. Translocation of ¹⁴C-endothall in 24 hours with a foliar application to <u>Poa</u> annua; whole plant on the left and radioautogram on the right.

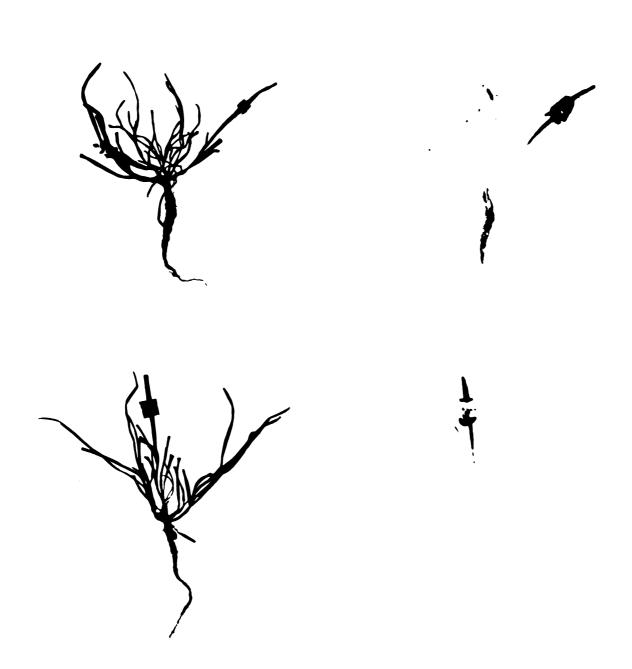


Figure 11. Translocation of ¹⁴C-endothall in 4 hours with a root application to Poa pratensis; whole plant on the left and radioautogram on the right.

Figure 12. Translocation of ¹⁴C-endothall in 4 hours with a root application to Agrostis palustris; whole plant on the left and radioautogram on the right.

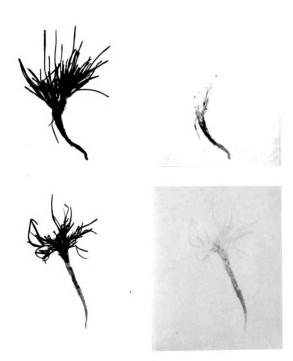


Figure 13. Translocation of ¹⁴C-endothall in 4 hours with a root application to <u>Poa annua</u>; whole plant on the left and radioautogram on the right.





to the sprayed portions of oat and bean plants. Maestri and Currier (1958) observed that endothall prevented basipetal movement of ¹⁴C-maleic hydrazide in barley. Apparently, the mobility of endothall in plants varies considerably among different species. Although there were some apparent differences in the rates of movement in the 3 turfgrass species, the herbicide is obviously quite mobile in both the symplastic and apoplastic systems of the plants. Hence, the observed selectivity of endothall is not readily attributable to translocation.

Metabolism: The initial metabolism study revealed no significant differences in the amounts of \$14CO_2\$ evolved from all species treated with \$14C-\$endothall\$ (Table 17). Since \$\frac{Poa}{annua}\$ took up substantially more endothall than did the other 2 species (Table 16), 2 alternative hypotheses appear feasible. If endothall degradation is dependent on herbicide concentration, then \$\frac{Poa}{annua}\$ annua metabolized the herbicide at a slower rate than did the other 2 species. Alternatively, if endothall degradation is dependent on detoxifying enzyme levels, and these levels are similar in all 3 species, then endothall metabolism would not contribute to selectivity.

Thin layer chromatographic analysis of extracts from treated plants revealed no significant differences in the nature of endothall metabolism. The predominant peak, in all cases, occurred with the 5 cm strip and exactly corresponded to the Rf value for endothall. This was assumed to be the parent herbicide. The endothall peak from the <u>Poa</u>

Table 17. Metabolism of $^{14}\text{C-endothal1}$ to $^{14}\text{CO}_2$ by 3 turfgrass species in 4 hours.

Species	14 _{CO2} released (dpm/g dry wt.)	$\frac{14_{\text{CO}_2} \text{ released}}{14_{\text{C-endothall applied}}} \times 10^3$
P. pratensi	<u>s</u> 7260 a ²	2.2 a
A. palustri	<u>s</u> 6634 a	2.0 a
P. annua	6610 a	2.0 a

 $^{^{\}scriptsize 1}$ Values are the means of 6 replications.

Means in column with common letters are not significantly different at the 5% level by the Tukey's Range Test.

annua extracts was approximately twice the height of those from Poa pratensis and Agrostis palustris, indicating substantially greater absorption of the herbicide by Poa annua (Figure 14). This was consistent with data from prior studies on root absorption (Table 16). Otherwise, there were no significant differences in the quantities of any metabolites when comparing the radioactivity curves of the 3 turfgrass species. Combustion and liquid scintillation counting of the acidified acetone-insoluble residues showed that the extraction was essentially complete for the ¹⁴C-label.

Although the rate of endothall metabolism may be somewhat slower in <u>Poa annua</u>, the nature of the metabolic transformations of endothall is essentially the same in all 3 species and cannot therefore contribute significantly to herbicidal selectivity.

Photosynthesis and Respiration: A sharp decline in photosynthesis (Figure 15) and a rapid increase in respiration (Figure 16) were observed for plants that had received the foliar-applied endothall treatments. This occurred within the first hour following application. The photosynthetic decline was particularly dramatic in Poa annua, especially in view of its relatively high initial photosynthetic activity. Although all of the plants had at least partially recovered by the end of the 48-hr test period, net photosynthesis in Poa annua was still substantially below the rate observed prior to treatment. The rapidity

Figure 14. Thin layer chromatographic separation of extracts from 3 turfgrass species treated with 14C-endothall.

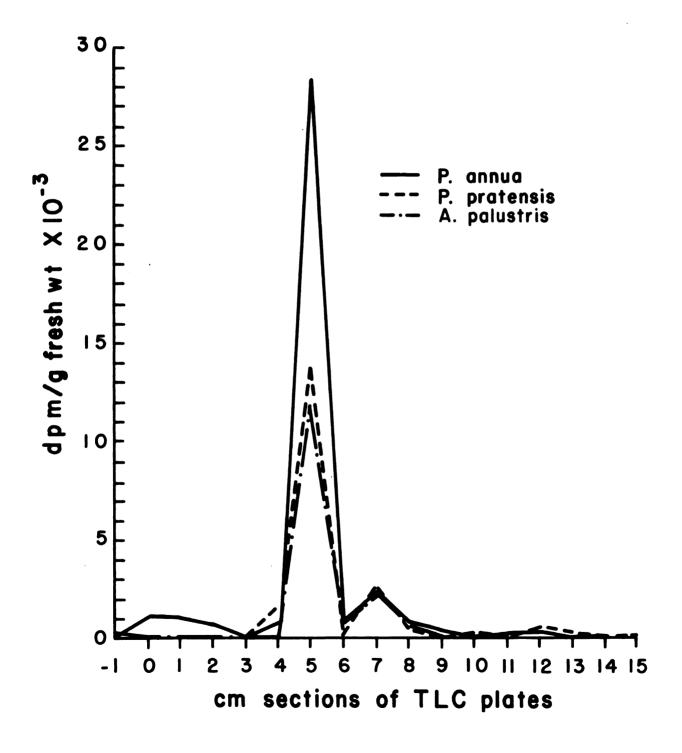
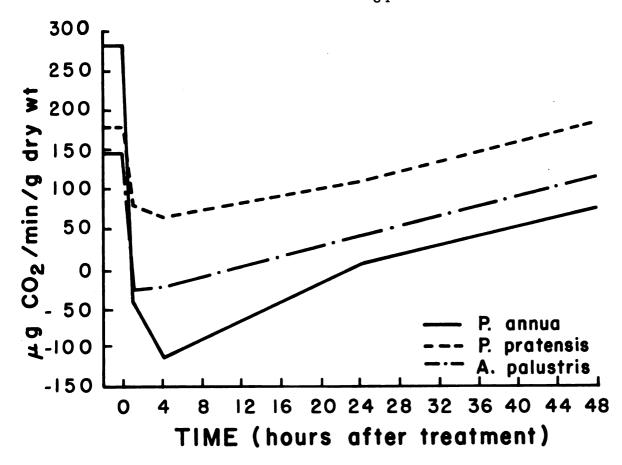
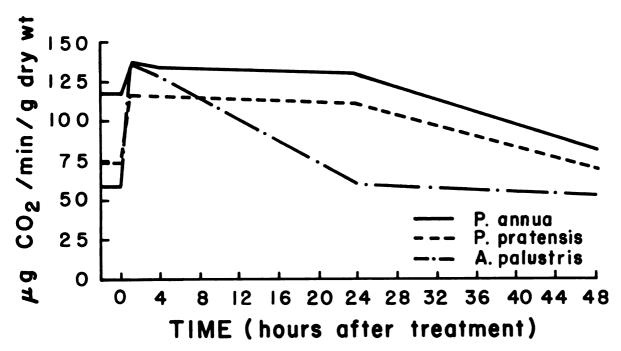


Figure 15. Net photosynthesis of 3 turfgrass species as affected by a 4.5 kg/ha foliar application of endothall.

Figure 16. Dark respiration of 3 turfgrass species as affected by a 4.5 kg/ha foliar application of endothall.





of this response suggests that endothall may produce a direct inhibitory effect on photosynthesis. Rakitin and Imamaliev (1959b) reported a decrease in the chlorophyll content of apple leaves following the application of endothall. Maestri (1967) observed grana disintegration and chloroplast shrinkage due to endothall. He further suggested that endothall might cause a general molecular disorder of cell membranes and a disturbance of cell compartmentation. It is conceivable that endothall may in some way disturb a wide variety of cell functions which then become manifested as the photosynthetic effects observed in this study. Furthermore, the differential growth suppression of Poa annua observed in the selectivity studies may be due to these differential effects on net photosynthesis.

The initial effects on respiration (Figure 16) were less pronounced in <u>Poa annua</u> than in the other grass species; however, respiration in <u>Poa annua</u> was significantly less at the end of the 48-hr test period than that observed prior to treatment. This was not true with the other grass species. The lower respiration rate of <u>Poa annua</u> following endothall treatment would presumably mean a lessened availability of energy for various synthetic processes upon which plant growth is dependent. Hence, this may also contribute to the differential growth suppression observed in the selectivity experiments.

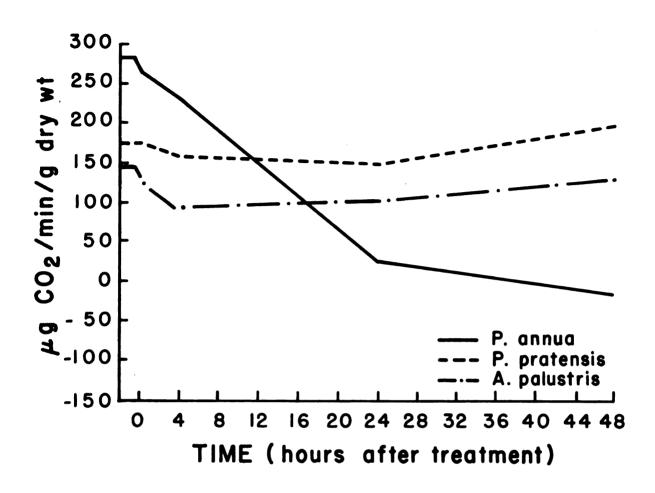
Root-applied endothall produced a different spectrum of responses in terms of net photosynthesis and respiration (Figures 17 and 18). The photosynthetic decline in the <u>Poa annua</u> plants was slower than that from foliar application of endothall, but the decline was continuous over the 48-hr test period. In contrast, the <u>Poa pratensis</u> and <u>Agrostis</u> palustris plants were only slightly affected in their photosynthetic activity.

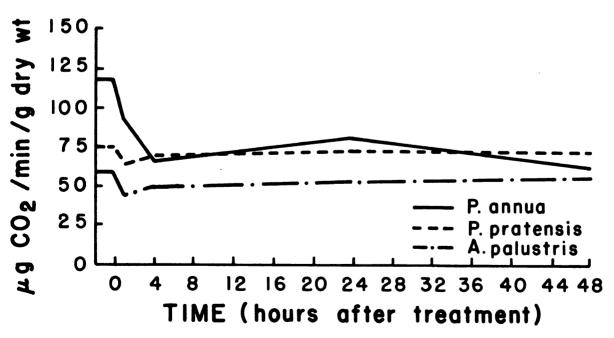
Respiration dropped significantly in <u>Poa annua</u> following endothall application to the roots and then remained essentially constant for the duration of the experiment. In contrast, the other grass species were only slightly affected. Furthermore, the dramatic rise in respiration in response to foliar application did not occur following application of endothall to the roots.

The difference in the photosynthetic response observed between foliar and root applications cannot be explained solely on the basis of translocation to the site of action. Results obtained from the radioautograms indicate very rapid translocation of the herbicide in these plants. These differences may be due to the different site of entry of the herbicide or to concentration differences. Furthermore, the superior selectivity observed with root applications may be due to other physiological effects. These are probably associated with the greater concentration of the herbicide in the roots of plants receiving root applications of endothall.

Figure 17. Net photosynthesis of 3 turfgrass species as affected by a 50 ppm endothall solution applied to the roots.

Figure 18. Dark respiration of 3 turfgrass species as affected by a 50 ppm endothall solution applied to the roots.





Transpiration: The plants that received a foliar application of endothall exhibited no significantly different water loss rate than the control plants over the 3-day test period, regardless of species. Poa annua plants that received the endothall root treatment lost significantly less water from transpiration than the untreated controls. This was apparent the first day following treatment with endothall (Table 18). A significant decrease in transpiration water loss was observed the second day after treatment with the Poa pratensis plants that had received endothall; however, the reduction of transpiration was much less than in Poa annua. The reduction in transpiration may have been due to an interference with water uptake by the roots or a restriction of stomatal opening in the leaves. The negative effect of the foliar application, however, suggests that stomatal function is not affected.

The transpiration data suggests some interference with root function following root-application of endothall.

This may be due to the apparently greater concentration of the herbicide in the roots from this application method.

Table 18. Transpiration water loss by 3 turfgrass species after foliar and root treatments with endothall.

Treatment	g water loss/g dry wt ^l			
rreatment	P. pratensis A. palustris P.	annua		
	1 day after treatment			
Control	19 a ² 30 a	27 a		
Foliar appl. (4.5 kg/ha)	19 a 29 a	27 a		
Root appl. (50 ppm)	16 a 28 a	16 b		
	2 days after treatment			
Control	56 a 77 a	74 a		
Foliar appl. (4.5 kg/ha)	56 a 76 a	74 a		
Root appl. (50 ppm)	38 b 66 a	35 b		
	3 days after treatment			
Control	89 a 123 a	121 a		
Foliar appl. (4.5 kg/ha)	87 a 121 a	122 a		
Root appl. (50 ppm)	60 b 101 a	51 b		

 $^{^{\}scriptsize 1}$ Values are the means of 6 replications.

² Means in each subcolumn with common letters are not significantly different at the 5% level by the Tukey's Range Test.

V. SUMMARY AND CONCLUSIONS

Annual bluegrass (<u>Poa annua</u> L.) is a weed when present in a turfgrass community because of its unsightly seedheads and its tendency to die out rather suddenly under conditions of mid-summer stress. Current turfgrass cultural and weed control practices leave <u>Poa annua</u> infestations in turf a widespread and perplexing problem for turfgrass managers.

Endothall may be a valuable herbicide when used as part of a program designed to replace <u>Poa annua</u> with desirable turfgrass species. Proper utilization of this herbicide must take into account its limitations as well as the conditions necessary for optimizing its selective activity.

The selectivity of endothall has been demonstrated with both foliar and root applications. In greenhouse studies, foliar sprays caused a growth suppression of <u>Poa annua</u>, but not of <u>Poa pratensis</u> and <u>Agrostis palustris</u>, at certain rates and frequencies of application. This occurred without causing discoloration or browning of the foliage.

In field experiments under high application rates, extensive foliar necrosis occurred in all species; however,

Poa pratensis and Agrostis palustris recovered due to their perennial nature of growth. This allowed Poa pratensis to invade areas previously infested with Poa annua.

Root applications of endothall resulted in greater selectivity between Poa annua and the perennial turfgrass species than the foliar sprays. A 50 ppm concentration of endothall was adequate to cause death of Poa annua plants

with no apparent effect on <u>Poa pratensis</u> or <u>Agrostis palustris</u>. The development of a granular formulation of endothall allowed root applications to be conducted in field turf. The lowest application rate adequate to selectively kill <u>Poa annua</u> was 9 kg/ha, but only under optimum conditions.

A number of factors were studied as possible bases for the selectivity of endothall. These were: the regenerative potential of perennial grasses; differential absorption, translocation and metabolism of endothall; and greater sensitivity of physiological systems to endothall in susceptible plants. Morphologically differing biotypes of Poa annua were shown to respond differently to endothall; a 50 ppm concentration of the herbicide supplied to the roots was effective in killing "annual" biotypes, while the "perennial" biotypes were only stunted from this treatment. This response was similar to the selectivity between the annual biotype of Poa annua and the perennial turfgrasses following applications of granular endothall in field studies, indicating that the regenerative capability of the perennial plants contributed to their tolerance.

Foliar applications of endothall revealed no significant differences in absorption by <u>Poa pratensis</u>, <u>Agrostis</u> <u>palustris</u> and <u>Poa annua</u> from a given area of leaf surface; however, <u>Poa annua</u> retained significantly more of the herbicide on its leaf surfaces, allowing for greater total absorption of endothall. Root absorption of endothall by

Poa annua was considerably greater than that observed for Poa pratensis or Agrostis palustris, although the amount translocated to the foliage was the same for all 3 species.

Poa annua does absorb more endothall from foliar and root applications. This difference accounted for approximately 50% of the selectivity from foliar applications and 20% from root applications based on endothall concentrations required for comparable phytotoxicity in the 3 species. The translocation of endothall from either foliar or root applications did not appear to contribute to selectivity.

No differences were observed among species in the amount of endothall metabolized to CO₂ or other breakdown products. Metabolism of a specific internal concentration of endothall was less in Poa annua than in the other grasses. Thus, metabolism makes only a limited contribution to selectivity.

The greater sensitivity of the physiological systems of <u>Poa annua</u> to endothall appears to be a major contribution to the basis of selectivity. Photosynthesis and respiration were substantially affected in all 3 grass species by endothall. Foliar applications produced a sharp decrease in net photosynthesis immediately after treatment, but <u>Poa pratensis</u> and <u>Agrostis palustris</u> recovered within 48 hours. Conversely, photosynthetic activity in <u>Poa annua</u> was still substantially below the level measured prior to treatment. Respiration increased sharply in <u>Poa pratensis</u> and <u>Agrostis</u>

palustris following a foliar application of endothall, but returned to normal within 48 hours. <u>Poa annua</u> responded with a slight increase in respiration, but this eventually dropped to a level substantially below that measured before application of the herbicide.

Experiments employing root applications of endothall resulted in the greatest selectivity between Poa annua and the 2 perennial grasses. Under these same conditions, the greater sensitivity of the physiological systems of Poa annua was the major contributor to the basis of selectivity. Root applications produced an entirely different spectrum of responses than observed with foliar treatments. Photosynthesis and respiration were only slightly affected in Poa pratensis and Agrostis palustris. Conversely, the photosynthetic activity of Poa annua dropped continuously over the 48-hour test period. This apparently signalled the death process, as foliar necrosis was observed within 72 hours after treatment and these plants did eventually Respiration also dropped significantly in the first 4 hours after treatment, but remained relatively stable over the next 44 hours.

Transpiration was not affected in any of the grasses following foliar application of endothall. Root application, however, caused significant reductions in transpiration in both Poa annua and Poa pratensis, but this response was considerably more pronounced in Poa annua.

Endothall may be used effectively for reducing <u>Poa</u>
<u>annua</u> infestations in Poa pratensis turf when applied as a

foliar spray in late summer, based on optimum conditions for selectivity reported in this study. Incorporation of a surfactant in the herbicide solution and the employment of relatively large spray volumes enhanced its effectiveness. The principal disadvantage of this approach is the temporary discoloration or browning of the turf associated with each application and the apparent necessity for repeated treatments.

Field applications did not result in measurable shifts in the percent species composition of <u>Poa annua</u>-infested turfs except when extensive foliar browning preceded the effect. Turfgrass discoloration was found to be dependent on the rate and season of application. Late summer and early fall applications were far more injurious to <u>Poa annua</u> than spring applications. This differential effect was attributed to an altered physiological condition of the plants preceding herbicide application.

Under these conditions, the regenerative capability of Poa pratensis allowed it to recover and invade the previously Poa annua-infested areas. Alternatively, root applications via the granular formulation of endothall offer considerable promise for removal of Poa annua from both Poa pratensis and Agrostis palustris turfs without causing discoloration of the perennial turfgrass species. The effectiveness of this approach apparently requires that the underlying soil be low in clay and organic matter, and that the turf be maintained under a frequent watering schedule prior to application of

the herbicide. The high developmental cost of the granular material might impose an economic limitation on its use.

The relatively simple structure and rapid biodegradability of endothall are desirable features in a herbicide for contemporary use. At a time when the contribution of pesticides to environmental pollution is open to public scrutiny, the employment of a short-lived herbicide in weed control deserves serious consideration.

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