## **Evaluation of EPTC as a Preplant Soil Treatment in Warm-Season Sod Production**

by

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Keywords: 2,4-D safening, enhanced biological degradation, EPTC, plant-back interval, turfgrass injury, warm-season sod production.

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#### **Abstract**

Field research was conducted at Auburn University Turfgrass Research Unit (AUTRU) Auburn AL, during 2008 to 2010 and Gulf Coast Research and Extension Center (GCREC) Fairhope AL in 2009, to evaluate EPTC as a preplant soil treatment in warm-season sod production and to determine if 2,4-D could be used as a potential safener for EPTC on three warm-season turfgrasses. For all three plant-back intervals, Palmetto St. Augustinegrass planted 1 week after treatment (1 WAT) at AUTRU showed significant dry weight differences among chemical treatments in both 2008 and 2009; similar treatment differences were not seen in the GCREC study. St. Augustinegrass planted 1 WAT produced similar or significantly higher dry weight, average stolon length or percent ground coverage than those planted back 2 WAT or 3 WAT at both locations. BK-7 zoysiagrass was less sensitive to EPTC applied alone or with dazomet. Plant-back interval comparisons suggested zoysiagrass could be planted back 1 week after EPTC or EPTC + dazomet treatment without receiving significant injury. Tifway bermudagrass planted at GCREC showed great sensitivity to EPTC. Previously described 2,4-D safening on corn and soybean against EPTC injury was not seen on bermudagrass or any of the turfgrass used at GCREC. Addition of the fumigant dazomet with EPTC did not adversely affected bermudagrass growth more than EPTC applied alone and this was also confirmed by the AUTRU cucumber bioassay, in which no significant dry weight differences could be found within EPTC and EPTC plus various rates of dazomet. GC-MS analysis of EPTC retention under field conditions indicated that EPTC applied at 7.84 kg ai/ha rate in July would be degraded to

very low concentration (around 0.01 ppm) in the top 7.5 cm soil within 7 days. GC-MS analysis also proved that a high rate of dazomet usage in previous years would increase enhanced biological degradation of EPTC the following year due to structural similarity.

Nomenclature: 2,4-D, 2,4-dichlorophenoxyacetic acid; corn, *Zea mays* L.; dazomet, tetrahydro-3,5,-dimethyl-2H-1,3,5-thiadiazine-2-thione; EPTC, ethyl *N, N*-dipropylthiocarbamate; GC-MS, gas chromatography-mass spectroscopy; soybean, *Glycine max* (L.) Merr.; St. Augustinegrass, *Stenotaphrum secundatum* (Walter) Kuntze., 'Palmetto'; Tifway bermudagrass, *Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy.; zoysiagrass, *Zoysia japonica* Steud., 'BK-7'. **Key words:** 2,4-D safening, enhanced biological degradation, EPTC, plant-back interval, turfgrass injury, warm-season sod production.

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## List of Abbreviations

AUTRU Auburn University Turfgrass Research Unit

DAT Days After Treatment

EPTC Ethyl *N*, *N*-dipropylthiocarbamate

fb Followed By

GC-MS Gas Chromatography-Mass Spectroscopy

GCREC Gulf Coast Research and Extension Center

WAT Weeks After Treatment

WALT Weeks After Last Treatment

#### **Literature Review**

Herbicide and Fumigant Control of Bermudagrass. Postemergence herbicide control of bermudagrass. Johnson (1992) conducted a study of common bermudagrass [Cynodon dactylon (L.) Pers.] control with fluazifop, fenoxaprop, sethoxydim and ethofumesate in a zoysiagrass (Zoysia spp.) turf at Griffin, GA. From the result of this study, following conclusions were made:

Sethoxydim. Sethoxydim applied at 0.25 lb ai/A in each of the four application dates (May 15, Jun. 13, Jul. 15 and Aug. 14) during 1989 and 1990 severely injured mixed common bermudagrass and zoysiagrass turf. The amount of injury varied slightly with date of application, but in most cases, was too severe to be acceptable. Sethoxydim reduced the population of common bermudagrass only slightly. Since zoysiagrass was severely injured after each sethoxydim application and common bermudagrass recovered quickly, sethoxydim did not have the potential to suppress common bermudagrass mixed with zoysiagrass.

Ethofumesate. Ethofumesate applied at 1.5 lb ai/A in each of the two applications during 1989 and 1990 severely injured zoysiagrass in common bermudagrass-zoysiagrass mixed turf. The cover of common bermudagrass increased from 35% to 78% and zoysiagrass cover decreased from 65% to 12% when final ratings were made in the spring of 1991. Therefore, ethofumesate could not be used for common bermudagrass control in zoysiagrass turf.

Fenoxaprop. When fenoxaprop was applied at 0.18 lb ai/A in four applications, common bermudagrass population was reduced from 35% to 23% after the first year, then the population was reduced to 3% after repeated applications. During this period, zoysiagrass turf density

increased at the same proportion common bermudagrass was reduced. Small amounts of common bermudagrass that survived after 2 years treatment will expand if left untreated during the third year. Fenoxaprop caused a maximum injury of 37% to the mixed common bermudagrass and zoysiagrass turf within 1 to 2 weeks after each application. However, the zoysiagrass turf fully recovered from injury within 3 weeks. Since the population of zoysiagrass increased and common bermudagrass decreased, zoysiagrass recovered from fenoxaprop treatment faster than common bermudagrass. No difference in common bermudagrass suppression was found when fenoxaprop was applied at 0.18 or 0.25 lb ai/A. Although fenoxaprop effectively suppressed common bermudagrass in this study, it did not control hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy.]. Therefore, correctly identifying bermudagrass species before selecting fenoxaprop for bermudagrass control in zoysiagrass turf is the key.

Fluazifop. When fluazifop was applied to mixed common bermudagrass and zoysiagrass turf, common bermudagrass was significantly suppressed after 1 year (from 35% to approximately 22% cover) and severely suppressed (3% to 7% cover) after 2 years. The suppression was similar when fluazifop was applied initially at 0.18 lb ai/A in May and followed by 0.09 lb ai/A in June compared to applied at 0.09 lb ai/A in four applications at monthly intervals. Therefore, there was no benefit in applying more than two fluazifop applications for common bermudagrass control. The bermudagrass suppression and zoysiagrass growth from fluazifop was similar to that with fenoxaprop. However, fenoxaprop caused a maximum of 37% injury to the mixed turf and injured turfgrass recovered within 1 to 2 weeks, while fluazifop caused a maximum injury of 78% and required 4 to 5 weeks to recover. Turfgrass injury was similar whether fluazifop was applied at 0.18 lb ai/A or 0.09 lb ai/A.

Johnson (1992) summarized that sethoxydim and ethofumesate could not be applied for common bermudagrass suppression when mixed with zoysiagrass. However, fenoxaprop and fluazifop could effectively suppress common bermudagrass, but multiple applications were needed for at least 2 years. Fenoxaprop should be applied at 0.18 lb ai/A at monthly intervals from mid-May until mid-August. Fluazifop should be applied at 0.18 lb ai/A mid-May and followed by another 0.09 lb ai/A application 1 month later.

In another study, Johnson (1987) tested several commonly used herbicides for hybrid bermudagrass control in tall fescue [Schedonorus phoenix (Scop.) Holub.], zoysiagrass and centipedegrass [Eremochloa ophiuroides (Munro) Hack.]. Fluazifop, sethoxydim, SC-1084 (Trophy), sulfometuron, metsulfuron were used in the study. Multiple metsulfuron applications had no effect on bermudagrass, but caused slight to moderate injury to centipedegrass, zoysiagrass and severely injured tall fescue. Multiple sulfometuron applications did not injure centipedegrass but caused slight injury to zoysiagrass and severe injury to tall fescue. Sulfometuron caused moderate to severe bermudagrass injury but did not prevent regrowth. SC-1084 at 0.25 lb ai/A resulted in variable bermudagrass control; the injury to tall fescue and zoysiagrass was slight to moderate, and to centipedegrass was moderate to severe. Fluazifop at 0.18 lb ai/A effectively controlled bermudagrass, but the number of applications varied from 3 to 6, depending on cultivar. Tall fescue tolerated fluazifop at 0.12 lb ai/A, while centipedegrass and zoysiagrass did not. Sethoxydim effectively controlled bermudagrass over a 2-year period. Centipedegrass tolerated sethoxydim, while the injury to zoysiagrass was moderate but produced severe injury to tall fescue.

Griffin et al. (1994) studied imazapyr in controlling common bermudagrass and evaluated herbicidal injury of imazapyr to hybrid bermudagrass, St. Augustinegrass [Stenotaphrum

secundatum (Walter) Kuntze.], centipedegrass and zoysiagrass, which were planted the year after initial imazapyr applications. Two repeat or single applications of imazapyr at 0.8, 1.1 or 1.7 kg ae/ha per application were made 1 June, 15 July, or 1 September, 1988 and were compared with glyphosate applied at 2.2 kg ae/ha on the same dates. All herbicide treatments provided 90% or greater control of common bermudagrass. A single July application of imazapyr at 1.1 kg ae/ha eradicated (100% control) common bermudagrass. Single applications of imazapyr caused less injury to the turf species than multiple applications at the same total rate. Less injury to desirable species occurred in 1989 than in 1988, which had limited rainfall. Bermudagrass and centipedegrass were injured less when planting was delayed until mid-summer the year following treatment, while spring plantings of St. Augustinegrass and zoysiagrass showed little or no injury from imazapyr applied the previous year. Their results indicated that common bermudagrass could be eradicated by a single application of 1.1 kg ae/ha of imazapyr, and desirable warm-season turfgrass could be successfully established in the following growing season.

Ferrell et al. (2005) evaluated imazapyr and glyphosate for chemical control of hybrid bermudagrass. Repeat applications were made 30 days after the first application beginning in mid-August each year. Imazapyr applied once at 0.56 or 1.12 kg ae/ha controlled hybrid bermudagrass (cv. 'Tifton 44') 88 and 97%, respectively, 52 weeks after treatment with no difference between rates observed. Additionally, imazapyr applied for 2 consecutive years totally controlled hybrid bermudagrass. Glyphosate isopropylamine salt and glyphosate trimethylsulfonium salt, applied for 1 year at rates of 4.2 or 1.7 fb (followed by) 1.7 kg ae/ha and 4.8 or 1.9 fb 1.9 kg ae/ha, respectively, provided between 70 and 78% control at 52 weeks after the last treatment (WALT). Hybrid bermudagrass control from either formulation of glyphosate

applied for 2 consecutive years ranged between 79 and 91% at 52 WALT. Compared to a 1-yr application program, either glyphosate formulation applied for 2 consecutive years did not significantly improve bermudagrass control at 52 WALT. The addition of fluazifop application at 0.42 kg ai/ha or clethodim at 0.2 kg ai/ha to glyphosate formulations did not significantly improve hybrid bermudagrass control relative to glyphosate applied alone. However, a tank-mix of clethodim plus either formulation of glyphosate applied for 2 consecutive years generally improved hybrid bermudagrass control relative to applications in only 1 year.

Waltz et al. (2001) tested clethodim for common bermudagrass control in centipedegrass and investigated centipedegrass tolerance to clethodim at four different locations in Georgia, South Carolina and North Carolina. In the first year, either a crop oil concentrate (COC) or non-ionic surfactant (NIS) was added to various rates of clethodim. In the second year, various application timings relative to centipedegrass green-up and rates of clethodim were investigated. Four monthly applications of clethodim at 0.14 kg ai/ha beginning at 60 to 100% centipedegrass green-up were best for season long bermudagrass control without significant centipedegrass injury. Furthermore, the addition of COC provided the best bermudagrass control with minimal centipedegrass injury. For centipedegrass sod producers, clethodim should be a possible chemical option for bermudagrass control.

Teuton et al. (2005) from University of Tennessee investigated hybrid bermudagrass control using combinations of glyphosate, fluazifop with the adjuvant ammonium sulfate (AMS). Herbicide treatments were: glyphosate alone, glyphosate plus AMS, glyphosate plus fluazifop, glyphosate plus fluazifop plus AMS. Their study results suggested that all treatments achieved over 80% control of hybrid bermudagrass 4 weeks after initial treatment (WAIT) to 12 WAIT treatment. At 8 WAIT treatment, all treatments almost provided 100% bermudagrass control.

And they also concluded that three applications significantly improved bermudagrass control by 12 percent over two applications at 12 WAIT. Moreover, adding fluazifop improved bermudagrass control at both 8 and 12 WAIT.

Preemergence herbicide control of bermudagrass. Fishel and Coats (1993) evaluated effect of several commonly used preemergence herbicides on bermudagrass root growth. They found that both prodiamine and oryzalin reduced 'Tifgreen' bermudagrass root growth in the upper 5 to 7.5 cm layer of a Bosket very fine sandy loam soil at 2 and 4 weeks after initial treatment. They also concluded that these herbicides reduced bermudagrass root weight in the 2.5-5 cm layer in both Bosket and Marietta soil. Prodiamine caused Tifgreen bermudagrass root weight decrease as low as 4 ppb by weight in the very fine sandy loam soil, while 8 ppb in the sandy clay loam soil.

McCarty (1996) evaluated atrazine, siduron, ethofumesate and flurprimidol for common bermudagrass control in St.Augustinegrass turf. Excellent control (> 95%) of common bermudagrass was observed after monthly applications of ethofumesate plus atrazine in March, April and May. Good control (80-89%) was observed with ethofumesate applied alone at the same months. Inconsistent and/or poor control (< 70%) of common bermudagrass was obtained with ethofumesate plus either flurprimidol or siduron. Additional treatments made in February and/or in November of the previous year did not increase bermudagrass control. St.

Augustinegrass turf quality was unaffected by ethofumesate plus atrazine. Turf quality was reduced 4 to 8 weeks after repeat applications of ethofumesate plus siduron. Ethofumesate plus flurprimidol combinations reduced turf quality for 8 to 12 weeks.

Walker et al. (2008) found that EPTC was very effective against common bermudagrass. Their study was carried out in Tallassee, AL. Treatments included: EPTC alone at 7 lb ai/A, dazomet alone at 100, 200, 300 and 400 lb ai/A and EPTC + dazomet at 100, 200, 300 and 400

lb ai/A combinations. Result was expressed as bermudagrass shoots number/250 feet<sup>2</sup>. Their result suggested that dazomet at 400 lb ai/A and EPTC 7 lb ai/A offered the same level of common bermudagrass control; the difference within these two treatments was not significant. For EPTC and dazomet combinations, dazomet at 400 lb ai/A + EPTC at 7 lb ai/A offered the best bermudagrass control, however, the difference between dazomet at 400 lb ai/A + EPTC 7 lb ai/A and EPTC 7 lb ai/A alone was not significant, which indicates EPTC may be a more economical way to solve bermudagrass infestation problem compared to dazomet and EPTC combination since the cost of dazomet is very high.

Fumigant control of bermudagrass. Methyl bromide, had been the primary broad-spectrum soil fumigant and general biocide for many years in agriculture production. However, its phase out officially began in January 1, 2005. It was one of the most commonly used, reliable fumigant available to many agronomic and horticultural crops, which effectively controls nematodes, weeds and pathogenic fungi. Earliest documented use of methyl bromide as a fumigant in turf is believed to have occurred at Greensboro, NC, when Greensboro Country Club renovated its putting greens back in the 1950's (Edwards and Barnes 1958).

Methyl bromide has been the most widely used fumigant in turf industry before it was phased out due to ozone depletion. Its predominant usage in turfgrass is to clean up existing weeds and injure weed seeds before turf establishment to ensure the genetic purity of the turf. This is especially important if the planting site is infested with weeds like bermudagrass, which will be very hard to eliminate in established turf. Some state sod certification agencies even require soil fumigant application prior to turf establishment and inspection of sod fields before issuing certification for the sod (Unruh et al. 2002).

Much research has been conducted with the objective of finding methyl bromide alternatives. Chemicals have included 1,3-dichloropropene, chloropicrin, dazomet, metam sodium, iodomethane and potassium azide or sodium azide (Gilreath et al. 2004a,2004b; Gilreath and Santos 2005a, 2005b; Locascio et al. 1997; Santos et al. 2006). Many studies focusing on methyl bromide alternatives have been conducted in vegetable and fruit crops such as tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) and strawberry (*Fragaria xananassa* Duch.), where nematodes and pathogenic fungi are primary threats in production. However, this is not the case in turf since turfgrass is an perennial crop, nematodes and pathogenic fungi can infest a turf after establishment, regardless whether preplant fumigation is performed or not. Usually, it is weeds which require preplant fumigation before turf planting (Unruh et al. 2002).

Walker et al. (2006) tested several fumigants for common bermudagrass control at Fairhope AL, Tallassee AL, Tuskegee AL, and Crossville AL. Treatments used in this study include: methyl bromide + chloropicrin at 400 + 47 lb ai/A; metam sodium at 320 lb ai/A with and without tarp; dazomet at 400 lb ai/A; sodium azide at 50 lb ai/A at Tallassee or 137 lb ai/A at Fairhope AL; 1, 3-dichloropropene at 542 lb ai/A and iodomethane + chloropicrin at 250 + 167 lb ai/A. Glyphosate at 4 lb ae/A and EPTC at 6.7 lb ai/A were also used in the study. Glyphosate at 4 lb ae/A was applied to half of each plot then tilled on June 13 (June 29 disk, July 12 chisel plow and disk, July 18 rototill) and fumigation was conducted in Crossville and Fairhope on July 18, 2001. Tarps were removed 9 days after treatment. The study at Tallassee was carried out in the summer 2006. Only two treatments were conducted at this location: EPTC at 6.7 lb ai/A and sodium azide at 50 lb ai/A. Their study results indicated that:

Crossville. Treatments provided 100% common bermudagrass control include:

Methyl bromide + chloropicrin, iodomethane + chloropicrin, 1, 3-dichloropropene, metam sodium no-tarp and no-glyphosate offered 80% control. Glyphosate and tillage provided 84% control and tillage alone provided 30% control.

Fairhope. Only three treatments offered 95% or greater common bermudagrass control: iodomethane + chloropicrin 97% control, 1, 3-dichloropropene 96% control, sodium azide 96% control. Glyphosate and tillage offered 50% control and tillage alone provided 44%. Adding glyphosate slightly helped increased bermudagrass control slightly.

Tallassee and Tuskegee. Sodium azide and EPTC used in both location for control of hybrid bermudagrass at Tuskegee and common bermudagrass at Tallassee. Complete control of hybrid and common bermudagrass was achieved at both locations. Yellow nutsedge (*Cyperus esculentus* L.) control averaged 94% at the Tallassee location.

In the end of this study, these researchers suggested that glyphosate pre-treatment followed by 1, 3-dichloropropene tarped or metam sodium + chloropicrin tarped were possible alternatives for warm-season sod producers as replacements for methyl bromide. However, there are several potential problems for these methyl bromide alternatives: ground water contamination from 1, 3-dichloropropene will limit it usage in some cases (U.S. EPA 1998, 2008). Moreover, plant-back interval may be 5 to 6 weeks under conditions of cool soil temperatures. The 1, 3-dichloropropene rate used in this study was very high (542 lb ai/A or 607 kg ai/ha) and it may be responsible for the excellent bermudagrass control provided by this chemical, but concomitantly, cost of fumigation would also dramatically increase. Noling and Becker (1994) reported that little weed activity was observed when 1, 3-dichloropropene was used at rates smaller than 266 kg ai/ha. In addition, metam sodium and 1, 3-dichloropropene have enhanced biological degradation problem after being used repeatedly (Dungan et al. 2001,

2003a, 2003b; Chung et al. 1999). The performance of metam sodium is not stable in soil since it requires soil moisture to produce the active ingredient methyl isothiocyanate (MITC), thus this process can be affected by soil conditions which is variable during a year. Applying metam sodium requires a certified commercial applicator license and some pesticide applicators in Alabama refused to apply metam sodium due to skin and nasal irritations. Requirements of personal protection equipment for applying metam sodium almost make fumigation impossible in extremely hot weather in the Southeast. Otherwise, cost of metam sodium application is expected to exceed that of methyl bromide by two or three times due to slower application speeds, multiple applicators and transportation costs (Unruh et al. 2002).

Sodium azide + EPTC combination has shown excellent bermudagrass control potential and a reasonable plant-back interval is around 3 to 4 weeks (Walker et al. 2005). Advantage for these chemicals is no tarp required since they can be applied with ordinary spray equipment. Although EPTC has been shown to undergo enhanced biological degradation after repeated use (Tal et al. 1989a, 1989b), it is believed that sodium azide may potentially alleviate this problem since it is a broad-spectrum biocide and EPTC enhanced biological degradation is primarily caused by soil microbes.

Methyl Bromide Alternative Research. Fumigants and weeds. Unruh et al. (2002) conducted a study to evaluate the weed control performance of iodomethane, metam sodium, 1, 3-dichloropropene, chloropicrin, dazomet and oxadiazon, either applied alone or in combinations. Their objective was to identify methyl bromide potential alternatives that could be used prior to turfgrass establishment. Experiments were carried out during 1998 and 1999 at two locations, Jay, FL and Arcadia, FL. Plots were sprayed with glyphosate at 4.2 kg ae/ha to kill the existing annual weed species and were harrowed and rototilled several times to prepare the seedbed for

fumigation treatments. Weed control data were collected by visual rating at 6 and 44 weeks after treatment at Jay and 3, 5 and 15 weeks after treatment in Arcadia.

Unruh et al. (2002) concluded that no EPA registered fumigant, or combinations of fumigant used in this study showed equivalent weed control efficacy as methyl bromide. Iodomethane was the most effective fumigant in this study and it controlled weedy grass species, sedge species and broadleaf weeds present at both locations. Previous research indicated that iodomethane performed more stable across different soil textures and was more effective than methyl bromide at low temperature, and this allowed this fumigant to be injected deeper into the soil since soil temperature decreases as the depth increases (Zhang et al. 1998). Potassium azide was as effective as methyl bromide in suppressing coastal bermudagrass ,*Cynodon dactylon* (L.) Pers., 'coastal'; broadleaf signalgrass ,*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster.; yellow and purple nutsedge, *Cyperus rotundus* L.; alexandergrass ,*Urochloa plantaginea* (Link) R. Webster.; tall morningglory, *Ipomoea purpurea* (L.) Roth.; sharppod morningglory, *Ipomoea cordatotriloba* Dennst. and various winter annual broadleaf weeds, but failed to control redroot pigweed (*Amaranthus retroflexus* L.). However, its potential for EPA registration is still unknown.

They also concluded that dazomet combinations provided acceptable control of coastal bermudagrass at Jay, FL, but control of common bermudagrass, alexandergrass and broadleaf signalgrass was not acceptable in Arcadia, FL. Meanwhile, poor sedge control (< 63%) was observed at both locations. Inconsistent control and sporadic failure of dazomet were observed in this study, partially because dazomet requires soil moisture to react with in order to produce the active ingredient methyl isothiocyanate. Failure to soil incorporate this material uniformly in the soil will decrease its weed control efficacy and this was observed in this study in the equipment

tire tracks and rototiller overlap which produced soil compaction. In addition, they found that metam sodium + chloropicrin tarped produced best weed control of all metam sodium treatments and was almost equal to methyl bromide in controlling weedy grasses and broadleaf species.

However, sedge control varied greatly between the two locations (56% at Jay and 79% at Arcadia). Also, like dazomet, sporadic failure was also observed with metam sodium + chloropicrin.

A study was conducted to compare effects of purple nutsedge control and tomato fruit numbers/yield after C-17 [1, 3-dichloropropene + chloropicrin in a 83:17 ratio (used rates were 325 kg ai/ha + 67 kg ai/ha in this study)] was applied alone or applied in combination with other herbicides (Gilreath and Santos 2005a). Herbicides used were pebulate at 4.5 kg ai/ha, napropamide at 4.5 kg ai/ha, metolachlor at 1.1 and 2.3 kg ai/ha, lactofen at 2.3 kg ai/ha, or flazasulfuron at 0.4 kg ai/ha. Pebulate achieved the best purple nutsedge control results, tomato fruit weights and numbers in all the herbicides used with C-17. Metolachlor also produced good purple nutsedge control and tomato fruit weight. These results corresponded with the study results of Gilreath et al. (2004c) that either in-bed or broadcast applications of 1, 3-dichloropropene + chloropicrin in combination with pebulate and napropamide were equally effective as methyl bromide against the sedges (*Cyperus* spp).

In another study (Gilreath and Santos 2005b), methyl bromide plus chloropicrin in a ratio of 67:33 at rates of 270 and 130 kg ai/ha was used as a standard of purple nutsedge control and tomato yield. Other herbicides and fumigants used in this study were: chloropicrin plus pebulate at 400 and 4.5 kg ai/ha; metam sodium plus pebulate at 485 and 4.5 kg ai/ha; dazomet plus pebulate at 950 and 4.5 kg ai/ha; and C-17 plus pebulate at 392 and 4.5 kg ai/ha, respectively.

After 12 weeks, methyl bromide plus chloropicrin provided a more effective control of purple nutsedge than other fumigants plus herbicides (10 plants/m<sup>2</sup> compared to 50 to 70 plants/m<sup>2</sup>). Comparing to methyl bromide plus chloropicrin, chloropicrin plus pebulate had a 14% lower tomato yield. No differences in tomato yield were observed with dazomet plus pebulate or C-17 plus pebulate compared to methyl bromide plus chloropicrin. However, metam sodium plus pebulate produced a 15% higher yield and 15% higher fruit weight than methyl bromide plus chloropicrin.

Motis et al. (2002) found that fumigants more effective controlled yellow nutsedge when tubers were imbibed than non-imbibed tubers. In 2000, both 1, 3-dichloropropene + chloropicrin in C-35 ratio and metam sodium alone offered 100% yellow nutsedge control. In 2001, metam sodium provided 100% yellow nutsedge control while 1, 3-dichloropropene + chloropicrin provided nearly total control with greater activity against imbibed than dry tubers. Yellow nutsedge control over 89% was obtained for 28 days after treatment with both fumigants. Also, irrigation prior to fumigant treatment dramatically improved yellow nutsedge control.

Hanson et al. (2007) evaluated cheeseweed (*Malva parviflora* L.) and bindweed (*Convolvulus arvensis* L.) seeds viability, weed emergence and biomass after Telone C-35 fumigant (61% 1, 3-dichloropropene plus 35% chloropicrin) application. On October 17, 2006, 499 kg ai/ha of Telone C-35 was applied at a 45-cm depth using a commercial Telone equipment with 51 cm spacing between shanks. Cloth bags containing field bindweed and cheeseweed seeds were buried 15 cm deep in each plot. Surface seal treatments included:

- 1. Control (moist soil per label instructions)
- 2. Manure + HDPE [composted steer manure incorporated into the soil surface at 12,350 kg/ha (5 ton/ac), then covered with high density polyethylene tarp (HDPE)]

- 3. Potassium thiosulfate (KTS) + HDPE (2:1 KTS/fumigant ratio, sprayed onto the soil surface in 1 mm water, then covered with HDPE tarp)
  - 4. Pre-irrigation (applied 34 mm water with sprinklers 4 days before fumigation)
- 5. Intermittent water seals (applied 13 mm water with sprinklers immediately following fumigation, plus an additional 4 mm at 12, 24, and 48 h)
- 6. Intermittent KTS applications (applied 2:1 KTS/fumigant ratio immediately following fumigation, and 1:1 KTS at 12, 24, and 48 h using the same amount of water as treatment 5.

The experiment results showed that there were no statistical differences in field bindweed and cheeseweed seed viability among the surface seal treatments. The fumigants did not reduce cheeseweed viability compared to the control; however, field bindweed viability was slightly reduced in all fumigated plots regardless of surface seal treatment. Emergence of resident weeds [primarily redmaids (*Calandrinia ciliata* (Ruiz & Pav.) DC. and annual bluegrass (*Poa annua* L.)] 2 months after treatment was strongly affected by both fumigation and surface seal treatment. In the fumigated plots, weed emergence was reduced by all surface seal treatments except for the control treatment. Pre-irrigation and the sequential KTS treatments reduced weed emergence even in the absence of fumigants. Weed biomass 3 months after treatment was variable but tended to be lowest in fumigated plots sealed with manure + HDPE, KTS 2:1 + HDPE, and sequential KTS.

Shrestha et al. (2008) tested fumigants performance on several common weeds in the United States. Treatments included a non-fumigated control; methyl bromide (98%) with high-density polyethylene (HDPE) film; iodomethane (50%) + chloropicrin (50%) with HDPE film; 1, 3-dichloropropene with HDPE film; 1, 3-dichloropropene (61%) + chloropicrin (35%) with HDPE film; 1, 3-dichloropropene (61%) + chloropicrin (35%) subsurface drip; and 1, 3-

dichloropropene (61%) + chloropicrin (35%) with virtually impermeable film (VIF). Weeds used in this study were common purslane, *Portulaca oleracea* L.; field bindweed; cheeseweed; Johnsongrass, *Sorghum halepense* (L.) Pers. and tall morningglory.

These researchers concluded that all the fumigants reduced the seed viability of common purslane, Johnsongrass, and tall morningglory but were not as effective on little mallow and field bindweed. Although total weed densities and the level of control provided by each fumigant differed between locations, weed density was generally reduced by all the fumigation treatments, compared to the non-fumigated control.

Klose et al. (2007) tested sensitivity of several weeds to Inline, a commercial fumigant formulation of 1, 3-dichloropropene (61%) plus chloropicrin (33%) by Dow Agrosciences, with logistic dose–response models. Among the weeds, the seeds of common purslane were most sensitive to soil fumigation with InLine (LC50 = 352 mmol/kg, LC90 = 583 mmol/kg), followed by common chickweed [*Stellaria media* (L.) Vill.] and common knotweed (*Polygonum plebeium* R. Br.) with LC90 values of 780 and 1636 mmol/kg soil, respectively. The seeds of cheeseweed mallow and redstem stork's bill (*Erodium cicutarium* L.) were not sensitive to fumigation up to the highest InLine dose of 19,520 mmol/kg soil.

Application and mulch. Candole et al. (2007) studied fumigant movements in soil and efficacy of drip-applied Inline (61:33 ratio) and metam sodium in plastic-mulched sandy soil beds. They found that higher Inline application rates resulted in higher concentration in the soil atmospheres at two pre-selected sites (10 cm below the emitter and 20 cm laterally away from the emitter). Differences occurred in fumigant concentration at these two sites: 1, 3-dichloropropene and chloropicrin concentrations were higher at 10 cm below the emitter than at 20 cm away from the emitter, which indicated that poor lateral movement of the fumigant occurred. This was

particularly obvious in sandy soils since in sandy soils, fumigants tend to drain more quickly than in loamy or silt soils. Methyl isothiocyanate, the breaking-down metabolite and active ingredient of metam sodium, was also found to have higher concentrations at 10 cm below the emitter than 20 cm away from the emitter and these facts resulted in poor pest control at the 20 cm site. Methyl isothiocyanate and 1, 3-dichloropropene + chloropicrin moved with the water more vertically than laterally, a movement that is typical in soils with higher sand content such as the soil in these studies (Ajwa and Trout 2004; Csinos et al. 2002).

Also in the study of Candole et al. (2007), combination of methyl bromide + chloropicrin was more effective at both sites (10 cm below the emitter and 20 cm laterally away from the emitter) in reducing the survival of pests and yellow nutsedge. The reason could be ascribed to methyl bromide having a higher diffusive mobility in soil due to its high volatility and its extremely low boiling temperature (4 C), and very high vapor pressure (184 kPa at 20 C) as well as higher Henry's constant (0.24 at 20 C). Comparing to methyl bromide, 1, 3-dichloropropene + chloropicrin and MITC have poorer mobility in soil due to higher boiling temperature (109-119 C); lower vapor pressure (2.5-3.3 kPa at 20 C) and lower Henry's constant (0.010-0.093 at 20 C) (Yates et al. 2003).

A uniform distribution of Inline was produced by spreading the fumigants in the liquid phase through the drip irrigation system (Ajwa et al. 2002; Gan et al. 1998). Higher gaseous concentrations of 1, 3-dichloropropene and chloropicrin in the soil air space, better distribution pattern of the chemicals, better pathogen control and higher strawberry yield were produced when InLine was applied with a large volume of water (61 L/m²) compared to a smaller volume of water (43 L/m²) (Ajwa and Trout 2004). The results of this study also showed that although concentration of fumigants in soil may appear insufficient because of large amount of irrigation

water applied with, water (> 40 L/m²) did help the diffusion of fumigants to a larger volume of soil and reduced fumigant volatilization losses during and after the application since soil pores were largely occupied by water. Meanwhile, the HDPE mulch used in this study did not effectively prevent or reduce fumigant volatilization and water was the only effective way to seal the fumigants within the soil (Ajwa and Trout 2004).

Soil surface tarps, such as standard high density polyethylene (HDPE), are commonly used to reduce fumigant emissions. The HDPE, however, did not effectively reduce 1, 3-dichloropropene emissions because of high permeability to this compound (Wang et al. 1999; Papiernik and Yates (2002). Another effective tarp to reduce emissions is virtually impermeable film (VIF), which has much lower permeability to more fumigants than HDPE. Virtually impermeable film, however, costs much more than HDPE (Wang et al. 1999; Noling 2002; Thomas et al. 2006).

In one study concerning fumigants 1, 3-dichloropropene and chloropicrin emission after application (Gao and Trout 2007), treatments were set as: 1. control (dry soil without tarp or water applications); 2. HDPE tarp over dry soil; 3. VIF tarp over dry soil; 4. pre-irrigated soil plus HDPE tarp (56 mm water was sprinkled on the surface 48 h before fumigation; this amount of water wet the soil to 30-cm depth and resulted in field capacity); 5. initial water application immediately followed by fumigation (19 mm water was sprinkled on the dry soil surface); 6. intermittent water applications, initial 19 mm water sprinkled immediately following fumigation plus 4.2 mm water sprinkled on soil surface at first sunset (8 h), first sunrise (22 h), noon (28 h), second sunset (32 h), and second sunrise (48 h) following fumigation.

The results of this study showed that among the surface seals, VIF and HDPE tarp over dry soil resulted in the lowest and the highest total emission losses among all the treatments,

respectively. Intermittent water applications reduced 1, 3-dichloropropene and chloropicrin emissions significantly more than using HDPE tarp alone. The initial water application also reduced peak emission and delayed emission time. Pre-irrigated soil plus HDPE tarp reduced fumigant emissions similarly as the intermittent water applications and also yielded the highest surface soil temperature, which may improve overall soil pest control.

Gilreath et al. (2004a) studied different effects of mulch type on fumigant Inline retention. Inline concentrations used in the study were 600, 800, 1000, 1200, 1400 ppm, respectively. Mulch types include: Pliant<sup>TM</sup> high barrier white on black, IPM Bromostop<sup>TM</sup> white on black, Pliant<sup>TM</sup> metalized and Klerk's<sup>TM</sup> green virtually impermeable film (VIF). During the first 3 days, Klerk's<sup>TM</sup> VIF and IPM Bromostop<sup>TM</sup> had the greatest fumigant retention. With 1400 ppm of Inline, Klerk's VIF<sup>TM</sup>, IPM Bromostop<sup>TM</sup> and Pliant<sup>TM</sup> metalized had 120%, 41% and 76% more Inline retention than Pliant<sup>TM</sup> high barrier white on black. With the same concentration, Klerk's VIF<sup>TM</sup>, IPM Bromostop<sup>TM</sup> and Pliant<sup>TM</sup> metalized produced 7, 2.5 and 4 times less nutsedge numbers, respectively, than Pliant<sup>TM</sup> high barrier, 12 weeks after fumigant injection.

Fumigants and soil microorganisms. effect of fumigation on microorganisms in unamemded and manure-amended soil. In one study conducted by Dungan et al. (2003b), researchers investigated the responses of microbial communities to the fumigants propargyl bromide and 1, 3-dichloropropene in unamended and manure-amended soil. The soil fumigants were applied at rates of 10, 100, and 500 mg/kg. After soil treatment, the metabolic activity was evaluated by monitoring the dehydrogenase activity (DHA). Propargyl bromide and 1, 3-dichloropropene initially inhibited DHA activity at 500 mg/kg concentration, however, recovery of the DHA only occurred in amended soil. Bacterial community level changes were identified by denaturing

gradient gel electrophoresis of polymerase chain reaction-amplified 16S rRNA fragments over a 12-week period of time. Numbers of bacterial communities were drastically reduced upon application of the fumigants, but reestablished more rapidly in the amended soil. In the propargyl bromide -treated soils, the diversity was higher in amended soil at all concentrations throughout the study, while in the 1, 3-dichloropropene treatments, the results were mixed. At 1, 4, 8, and 12 weeks after fumigation, major bands were excised from the gels and the DNA was cloned for sequence analysis. The bacterial communities in the fumigated amended soils were dominated by *Streptomyces* spp., other genera of *actinomycetales*, including *Frankia*, *Cytophagales*, *Actinomadura*, and *Geodermatophilus*, and a number of unidentified bacteria.

In another study (Ibekwe et al. 2001), compost effects on microbial diversity in soil with repeated 1, 3-dichloropropene applications were evaluated. After 8 weeks of incubation with repeated applications of 1, 3-dichloropropene, volatilization fluxes were much lower for compost-amended soil than that of the unamended soils, which indicated compost had an effect on increasing the degradation of 1, 3-dichloropropene. In this study, denaturing gradient gel electrophoresis (DGGE) profiles of the PCR-amplified region of 16S rDNA genes were involved to identify dominant bacterial populations in the fumigant-degrading soil. The DGGE results indicated that specific bacterial types had been enriched, more diverse fingerprints were found in the bacteria community of the compost-amended soil compared to the unamended soil. Two clones, E1 and E4, were unique in all compost-amended soils and related to strains of *Pseudomonas* and *Actinomadura*, respectively.

Fumigants and soil microorganisms. effect of fumigation on soil microorganisms. Stromberger et al. (2005) have found that fungal populations and the activities of acid phosphatase as well as arylsulfatase were more sensitive to fumigations (1, 3-dichloropropene, iodomethane and

propargyl bromide) than total microbial biomass, microbial respiration, nitrification, and activities of dehydrogenases and P-glucosidase. Their research results suggested that fumigation with methyl bromide plus chloropicrin eliminated soil-borne fungal pathogens in soil and reduced culturable fungal populations up to 4 weeks after fumigation. Soil microbial respiration decreased after fumigant application and was the least (> 40% reduction compared to the control) in the propargyl bromide treatment 1 week after fumigation, while soil microbial biomass carbon was not affected by fumigation. The activities of acid phosphatase and arylsulfatase were generally lower in fumigated soils over the whole 30- or 37-week study, while those of P-glucosidase and dehydrogenase were lower up to 4 weeks after fumigation. Potential nitrification rates were substantially reduced (> 55% reduction relative to the control) by the fumigants, but rates recovered towards the end of this study.

Fumigants and soil microorganisms. microbial and abiotic degradation of fumigants. Hydrolysis is the first step for degradation of the fumigant 1, 3-dicholorpropene in water and soil (Guo et al. 2004). Experiments were conducted to investigate effects of various environmental factors on the hydrolysis reaction. Cis-, trans-1, 3-dicholorpropene and their isomeric mixtures were mixed into water solution and soils (coarse-loamy soil), then incubated under different conditions. Hydrolyses of the chemical in water and soil were evaluated based on its residual amount and the Cl<sup>-</sup> release, respectively. Fumigant 1, 3-dicholorpropene hydrolyzed rapidly in deionised water, with a half-life of 9.9 d at 20 C. The hydrolysis was pH dependent while acidic pH inhibiting hydrolysis and alkaline pH increasing the reaction. Other factors such as isomeric difference, photo irradiation, suspended particles, untamed microbes, and small amounts of solutes had almost no effect on the reaction. In soil, 1, 3-dicholorpropene hydrolyzation rate decreased with the initial concentration but increased with soil moisture content. At 20 C, half-lives of 1, 3-

dicholorpropene with application rates of 10 and 0.61 mg/g in 10% moisturized soil were 35.7 d and 15.2 d, respectively. The isomeric difference, soil particle size and mineralogy had little effect on the reaction. Microbial contribution was not important at beginning of the incubation, but became important when soil microorganisms adapted to the fumigant. These results suggested that pH, tamed microbes, soil moisture, and organic amendment are related to the rate of 1, 3-dicholorpropene degradation.

Enhanced degradation of 1, 3-dichloropropene has been studied by many scientists (Chung et al. 1999; Ou et al. 1995; Smelt et al.1989, 1996; Verhagen et al. 1996). Other fumigants, such as metam sodium, also partially loses effectiveness due to enhanced degradation after repeat applications (Smelt et al. 1989; Verhagen et al. 1996; Chung et al. 1999). Many factors may be responsible: soil moisture content, soil pH, soil texture, soil organic matter content, soil microbial population and communities, concentration of nucleophiles, etc. (Gan et al. 1999; Verhagen et al. 1996; Dungan et al. 2001). Ou et al. (1995) found that (E)-1, 3-dichloropropene in an enhanced soil in Florida was degraded faster than (Z)-1, 3-dichloropropene. Microorganisms were mainly responsible for enhanced degradation of pesticides in soils. They also found that in 1, 3-dicholorpropene-treated and-untreated soils, mineralization decreased as the depth of soil increased, suggesting oxygen is required during the degradation process.

In one study, researchers at the University of Minnesota (Zhang et al. 2005) evaluated relative contribution of microbial degradation to the total degradation of MITC and chloropicrin. Fresh, sterilized and autoclaved soils from two locations (Byromville, GA and Hayward, WI) were used in the incubation study. Fumigation concentrations were set as 195, 390, 785 kg ai/ha. Their experiment results suggested that microbial degradation accounted for about 60% of the

total degradation of MITC and 40 to 80% of chloropicrin in the two incubation soils. Their experiment results also showed that the degradation of MITC and chloropicrin was independent of soil water content until it exceeded 15% by weight.

Furthermore, from their result, it was obvious that half lives of MITC and chloropicrin, in most of the fumigant concentrations, lasted longer in either fresh or fumigated Hayward soil than in fresh or fumigated Byromville soil (except for 390 kg ai/ha fumigant application in fresh soil incubation). Different soil physical properties and microbial communities may have been responsible for this response.

Zheng et al. (2003) investigated the degradation of chloropicrin, 1, 3-dichloropropene and their mixture in fresh and sterile soils. Scientists found that degradation of low concentrations of chloropicrin in fresh soil was accelerated initially if 1, 3-dicholorpropene was presented, whereas the addition of chloropicrin reduced the degradation rate of *trans*-1, 3-dicholorpropene. They also found that the degradation of both fumigants was significantly enhanced in soils amended with ammonium thiosulfate (ATS) and sodium diethyldithiocarbamate (Na-DEDTC) compared with unamended soil. Abiotic transformation accounted for 80% of the total 1, 3-dicholorpropene degradation in amended soils. Furthermore, *trans*-1, 3-dicholorpropene was more susceptible to microbial degradation while *cis*-1, 3-dicholorpropene changed more quickly in abiotic transformations.

These researchers also suggested, after applications of 1, 3-dicholorpropene and chloropicrin mixture, the fumigant compounds appeared to behave relatively independently in their degradation, indicating that application of fumigant mixtures may have little effect on the environmental fate and efficacy of these compounds.

EPTC and Thiocarbamates Enhanced Biological Degradation Research. After losing methyl bromide, an effective tool for bermudagrass control in turfgrass establishment, many research studies have been conducted with the objectives of finding possible fumigant alternatives for methyl bromide (Gilreath et al. 2004a,2004b, Gilreath and Santos 2005a, 2005b; Locascio et al. 1997; Santos et al. 2006). However, there has been no fumigant or fumigant combinations that performed as well as methyl bromide for bermudagrass control. Many technical difficulties render these fumigant alternatives either problematic during the application or too expensive when comparing to the benefits provided (Unruh et al. 2002).

Previous studies have confirmed that EPTC (Eptam) is very effective against common bermudagrass (Walker and Belcher 2006, 2008) and it is a cheap option costing around \$40/acre. However, enhanced biological degradation of EPTC may reduce weed control efficacy of this herbicide after repeated use (Tal et al. 1989a). So, relevant studies and previous literatures concerning this issue will be reviewed next.

EPTC (ethyl *N*, *N*-dipropylthiocarbamate) is a selective herbicide of the class thiocarbamates (also known as carbamothioates). It is widely used to control annual grassy weeds, perennial weeds, and some broadleaf weeds in beans, forage legumes, potatoes, corn, and sweet potatoes (Senseman 2007) and it is most effective against germinating seeds. EPTC is usually incorporated into the soil immediately after application either by mechanical tillage or by overhead irrigation, due to its very high volatility (Senseman 2007). EPTC is available as an emulsifiable concentrate and granular formulations.

Molecular Structure of EPTC:

(Chemblink)

www.chemblink.com/products/759-94

4.htm

EPTC is moderately mobile in soil and has a moderate leaching potential into the groundwater. It is soluble in water (375 ug/ml), and binds only weakly to soil particles (Koc = 280 g/ml). EPTC binds to the dry soils but is readily removed from clay particles by soil water (Saltzman and Yaron 1986). The amount of leaching decreases as clay or organic matter content of the soil increases. In sandy soils inside glass columns, EPTC moved to a depth of 9 to 15 inches when 8 inches of water was applied. When the same test was performed on loam and clay soils, the herbicide moved to a depth of only 3 to 7 inches. EPTC is a light yellow liquid with an amine odor which is the characteristic of the thiocarbamates. Repeat uses of EPTC will cause enhanced microbial degradation of this herbicide (Senseman 2007).

# Soil Types, pH and Organic Matter Influence on Thiocarbamates Degradation.

Thiocarbamates degradation can be influenced by soil type, pH and organic matter content of soil. Some researchers suggested that high pH and low organic matter content resulted in greater enhanced biodegradation and poor weed control (Gray and Joo 1985). This was inconsistent with other researchers' finding. In a study conducted at Dothan, Varina, and Wagram, SC concerning thiocarbamates degradation in butylate-history soils (McCusker et al. 1988), thiocarbamates degradation was highest in Dothan soil followed by Varina and Wagram soils. Organic matter contents in Dothan, Varina, and Wagram soils were 1.1, 0.9, and 0.7%, respectively. Highest degradation occurred in the soil with highest organic contents. Lode and Skuterud (2006) reported that the degradation of EPTC and consequently, its phytotoxicity to ryegrass (*Lolium* 

perenne L.) was greatly influenced by the pH in the three soil types tested. On raising the pH from approximately 5 to 7, the microflora and the EPTC degradation in the soil increased and the phytotoxic period was shortened by 2-3 weeks in the greenhouse experiments. A similar increase in the degradation of EPTC was also found by adding manure.

Obrigawitch and Tobias (1982a) tested EPTC degradation rate in soils with organic matter contents of 1.0% and 2.4%. Degradation of EPTC was faster in the 2.4% organic content soil than the soil with 1.0% organic matter content. It was possible that insufficient carbon source in soil resulted in limited numbers of microorganisms and slower herbicide degradation.

This hypothesis was also proposed by Bean et al. (1990) from their study involved with the extenders dietholate, fonofos, and SC-0058 [S-ethyl di-(3-chlorallyl) carbamothioate]. The extenders (SC-0058 and dietholate) seemed to be more effective in slowing biodegradation of EPTC in Scottsbluff soil than Clay Center soil. One explanation was the difference in organic matter content. The organic content in Clay Center soil was higher than that of the Scottsbluff soil, therefore, larger microorganism populations were believed to exist in the Clay Center soil. This fact might leaded to faster enhanced biodegradation of EPTC and which overcame the effect of the extenders. It was also possible that some other soil factors contributed to different degradation rates of EPTC.

**Temperature Effect on Enhanced Biodegradation of Thiocarbamates.** *Different temperature effect on EPTC degradation.* In a research studying the influence of temperature, moisture, previous application history and herbicide extenders on degradation of thiocarbamate herbicides, temperatures below 15 C, especially below 5 C, extended EPTC persistence in soil exhibiting thiocarbamates enhanced degradation, while slightly increased persistence of EPTC in non-thiocarbamates history soil. It was very likely that microbial enzyme activities required to

degrade thiocarbamates were inhibited by low temperature. At 25 C, EPTC degradation was fastest among all the temperatures tested in this study (Obrigawitch et al. 1982b). *Storage effect on thiocarbamates degradation*. Laboratory experiments were conducted to evaluate butylate and EPTC degradation in butylate history soils after cold storage at 4 C for a certain period of time. No significant differences in degradation of butylate and EPTC were found due to the length of cold storage of soils (0, 1 or 2 years). Enhanced degradation of butylate and cross-adaption for EPTC were not affected by cold storage. Storage of soils up to 2 years did not adversely affect these results (McCusker et al. 1990).

The ability of microorganism to cause enhanced EPTC biodegradation was retained in soils stored 1 year at ambient temperature. The lag phase before rapid EPTC degradation in a fine sandy loam soil after storage was 27 to 30 days compared to a fresh soil sample. The lag phase was only 3 days in a heavy silt loam (Lee et al. 1984). This difference may in part be due to different organic contents of these soils.

Harvey (1987) found that the ability of soil to maintain enhanced biodegradation of EPTC was reduced or lost by soil storage at 15 C or 25 C for 12 or 6 months, respectively, in a Wisconsin soil. Enhanced biodegradation of EPTC was not affected by storage of the Plano soil at 5 C for 12 months. Thus, temperature may play an important role in retaining the potential of enhanced biodegradation ability of EPTC history soil.

Although enhanced biodegradative ability of whole soil remained after 2 years of storage at 4 C, individual bacterial isolates lost their degradative capabilities after 3 to 6 months storage on agar slants at 4 C (Mueller et al. 1988). Thus, soils may provide a protective mechanism for the degradative genes of bacteria which might be lost in a pure culture.

**Volatilization of Thiocarbamates Herbicide.** Volatilization, a physical process that depends upon both pesticide and soil properties, dominates the performance and persistence of thiocarbamate in the soil (Roeth 1986). About 40% of the total <sup>14</sup>C-EPTC was lost through volatilization during the first 3 days of the incubation in two soils and thiocarbamate application history did not affect volatilization (Tal et al. 1989b).

Different degradation rate of thiocarbamate may be responsible for different volatilization rates found in a study involved with butylate-history soils in South Carolina (McCusker et al. 1988). In this study, <sup>14</sup>C-labeled butylate, cycloate, EPTC, pebulate and vernolate were applied to 3 years butylate-history soils that received three to eight butylate applications under field-conditions. Cycloate had the highest volatilization of all the thiocarbamate herbicides in this study and volatilization was similar between butylate-history and non-butylate-history soil. However, cycloate had the lowest enhanced biodegradation among all the thiocarbamates in this study, which means butylate-adapted microorganisms were not cross-adapted for cycloate. Low volatilization loss of EPTC and butylate was found in both butylate-history and non-butylate-history soil, probably due to greater enhanced degradation of EPTC and butylate by soil microorganisms, which resulted in less available EPTC and butylate to volatilize from the soil (McCusker et al. 1988).

Enhanced Biological Degradation: Bacteria (*Rhodococcus*). *Rhodococcus*. Genus *Rhodococcus*, is believed to have a strong ability to degrade EPTC and is also able to degrade triazine-family herbicides like atrazine, simazine and propazine (Behki and Khan 1994). In one study (McClung et al. 1994), microorganisms capable of degrading EPTC were isolated from three soils with or without EPTC history in pure culture systems. All EPTC-degrading isolates belonged to the genus *Rhodococcus* (identified as JE1, DE1 and TE1). The starting

concentration of 50 ug/ml EPTC was degraded by isolates JE1 and DE1 over an 8-12 h period with a simultaneous increase in the number of bacterial cells determined by increasing turbidity of the cultures. These results are consistent with studied results of Tam et al. (1988), who reported degradation of 30 ug/ml EPTC over a 8-h period resulted in similar increases in cell density for their isolate, designated TE1, which was also a *Rhodococcus*.

Lag phase of degradation. In a study that evaluated resting cell suspension of *Rhodococcus* isolate TE1 for growth on different nutrient environments, there was a lag period in EPTC degradation observed when glycerol-grown TE1 cells were used for the resting cell suspensions, but there was no lag when EPTC-grown cells were used (McClung et al. 1994). This indicates that the genes encoding the EPTC degradation enzyme(s) in these isolates are induced by EPTC or some metabolites of EPTC.

Loss of degradative ability. McClung et al. (1994) reported that, for the three new EPTC isolated strains obtained by pure culture systems from soil with or without EPTC history, there was a high spontaneous loss of EPTC-degradative capability in the three newly isolated *Rhodococcus* strains when they were grown in nutrient broth for a short period. Between 77% and 91% of the colonies isolated from the nutrient broth exhibited poor or no growth on EPTC plates. Treatment with the plasmid curing agent, acridine orange, or an elevated temperature (32 C) did not affect the frequency of loss of EPTC-degrading ability of the new strains.

Other microorganisms responsible for EPTC enhanced degradation. Lee (1984) reported that 29 EPTC-degrading fungal isolates belonged to 11 taxa and only one, *Epicoccum purpurascens*, was not a common soil specie. Within the bacterial isolates, nine of 110 bacterial isolates degraded significant amounts of EPTC. However, after 15 months storage at 4 C on nutrient agar slants, none of these bacterial isolates were effective degraders, while most of the fungal isolates

remained the ability to degrade EPTC at a fast rate. One explanation for the loss of degradation ability of bacteria is that genes for degrading EPTC may be located on plasmids which are lost during prolonged storage and sub-culture.

**Extenders.** Dietholate and 2-methoxyethylmercury chloride. Tal et al. (1989b) tested effects of different soil sterilization methods, which included both physical and chemical sterilization, on the degradation of EPTC in a vernolate-history soil. They found that antimicrobial agent 2-methoxy-ethylmercury chloride strongly inhibited EPTC degradation during the entire incubation. The result suggested that it was possible that this antimicrobial agent 2-methoxy-ethylmercury chloride created completely 'sterilized' soil and suppressed a wide range of microorganisms. So the inhibition effect was almost equal to that provided by autoclaving or gamma irradiation.

Moreover, these researchers also found that dietholate was a very effective EPTC extender. It strongly inhibited microbial degradation of EPTC possibly by inhibiting enzyme needed for degradation, rather than inhibiting the growth of soil microorganisms. However, multi-years usage of dietholate and EPTC resulted in re-occurrence of EPTC enhanced degradation (Roeth 1986).

Obrigawitch and Tobias (1982a) also proposed the idea that dietholate had the ability to inhibit the hydroxylation pathway, which was believed to be the dominant pathway of EPTC in soil showing enhanced degradation; dietholate slowed down degradation of EPTC in soils exhibiting enhanced degradation (Obrigawitch and Tobias 1982a).

Organophosphate: effect of OP insecticides on the degradation of EPTC by Rhodococcus TE1.

Behki and Khan (1991) tested effects of organophosphorus insecticides on the EPTC degradation by Rhodococcus TE1 and they concluded that the inhibitory effect of organophosphorus

insecticides on TE1 EPTC degradative ability was largely due to inhibition of TE1 growth. Malathion, phorate, parathion, fonofos, and isofenphos were used in this study to inhibit EPTC degradation by *Rhodococcus* TE1: malathion and phorate had no effect on TE1 strain growth and EPTC degradation. Fonofos and isofenphos inhibited EPTC degradation in the first 24h, but inhibition diminished after this time. It was believed that rapid loss of their inhibitory ability was due to quick microbial degradation of these insecticides themselves. Parathion seemed to have the best inhibitory effect on EPTC degradation and its inhibition effect also lasted longer than fonofos and isofenphos. Dramatic inhibition of EPTC degradation by parathion was longer than 48h. Meanwhile, parathion had a strong inhibitory effect on butylate degradation. Moreover, parathion's inhibitory effect on the growth of TE1 and EPTC degradation was almost instant. It inhibited TE1 growth immediately after been applied to TE1 culture.

Behki and Khan (1991) also found that parathion did not have the ability to inhibit the growth of *Pseudomonas putida*, *Escherichia coli*, a strain of *Flavobacterium*, or *Arthrobacter oxydans*. Nevertheless, it did dramatically inhibit the growth of *Rhodococcus* TE1, TE3, and *Rhodococcus*. *erythropolis*. TE3 and *Rhodococcus erythropolis* were not able to degrade EPTC, so they suggested that the inhibitory effect of parathion on bacteria growth was probably speciesspecific and was not related to the ability of strains to degrade EPTC.

Organophosphate: degradation of parathion by TE1 cells. Behki and Khan (1991) tested the parathion degradative ability of the strain *Rhodococcus* TE1. Only small amounts of parathion degradation occurred during the first 2 days, and then, degradation occurred faster at 27%, 46%, and 66% of the total parathion applied by the end of 3rd, 5th and 7th days, respectively. TE3 also showed the ability to degrade parathion with 21%, 35%, and 52% degradation after 3, 5 and 7 days, respectively. The results of this study suggested that paraoxon, the metabolite product of

parathion, also has the ability to inhibit TE1 to degrade EPTC. However, paraoxon is short-lived in the environment, and it is further hydrolyzed to diethyl-phosphoric acid and p-nitrophenol rapidly. Researchers found that p-nitrophenol had no effect on microbial degradation of EPTC (Fuhremann and Lichtenstien 1980).

Fungicide. Fungi may play a more important role than bacteria in the degradation of EPTC. Average of 67%-86% of fungal isolates were effective degraders compared to only 8% of bacterial isolates (Lee 1984). However, this was inconsistent with the study results of Tal et al. (1989). In this study, cycloheximide, an antifungal antibiotic, had almost no obvious effect on the <sup>14</sup>CO<sub>2</sub> evolution from <sup>14</sup>C-EPTC, while the antibacterial antibiotic chloramphenicol significantly reduced <sup>14</sup>CO<sub>2</sub> release during the whole experiment. These facts suggested that soil bacteria were mainly responsible for enhanced biodegradation of EPTC in soil. Rache and Coats (1987) used chloramphenicol and cyclohexinide to quench microbial degradation of the insecticide isofenphos, and their results also indicated that bacteria, not fungi, were primarily responsible for rapid degradation of this insecticide.

*SC-0058*. SC-0058 [*S*-ethyl di-(3-chlorallyl) carbamothioate] appears to inhibit biodegradation of thiocarbamates by a different mechanism since after SC-0058 treatment, weed control was significantly improved in soils with EPTC + dietholate and EPTC + fonofos history (Bean et al. 1990). SC-0058 seems to be an effective EPTC extender and in some cases it may perform better than dietholate or fonofos.

In this study with SC-0058 (Bean et al.1990), residual amounts of dietholate and SC-0058 were analyzed in soils exhibiting enhanced degradation of EPTC. The results indicated that dietholate and SC-0058 retention was not affected by repeated uses; they did not experience self-

enhanced degradation like the organophosphate extenders. It is possible that both of these extenders are enzyme-inhibitors, inhibit enzymes needed for EPTC degradation.

Enhanced Biodegradation of EPTC. Fate of EPTC in soils exhibiting enhanced biodegradation. In one study concerning the fate of <sup>14</sup>C-EPTC in soils exhibiting enhanced biodegradation, soil samples were taken from a field that was treated with vernolate for 6 years consecutively (Tal et al. 1989b). Then, 10 mg/kg <sup>14</sup>C-EPTC (0.5 μCi/50g) was added to each soil sample. In the first 3 days of incubation, no significant differences of organic phase <sup>14</sup>C-EPTC were detected within vernolate history soil and the control (soil sampled from an adjacent field without thiocarbamate history.). This was possibly due to volatilization of EPTC at this early phase, in which 35% and 32% of the total <sup>14</sup>C-EPTC in the control and vernolate history soil, respectively, dissipated in the vapor form; however, in the next 6 days, the vernolate-history soil exhibited a greater EPTC degradation rate.

At the 9<sup>th</sup> day of the incubation, only 16% of the <sup>14</sup>C-EPTC still remained in the organic phase in the soil, compared to 43% of <sup>14</sup>C-EPTC remaining in the control soil. On the 15<sup>th</sup> day of the incubation, there was no organic phase <sup>14</sup>C-EPTC detected in the vernolate history soil, compared to 28% remaining in the control soil; 56% of total <sup>14</sup>C found in CO<sub>2</sub> form, 37% of <sup>14</sup>C found as <sup>14</sup>C-EPTC vapor in vernolate-history soil. The amount of <sup>14</sup>C detected in the polar phase and non-extractable form was very limited (< 5%) and the total <sup>14</sup>C recovered amounted to 93% and 98% in the non-vernolate history and vernolate-history soils, respectively.

Moorman (1988), using <sup>14</sup>C-MPN (most-probable-number) technique, has confirmed that increased rates of metabolism of EPTC were apparently responsible for the enhanced degradation of EPTC, rather than increased populations of degraders (Moorman 1988). However, Mueller et al. (1989) reported greater numbers of actinomycete capable of degrading butylate in

soils previously treated with butylate or vernolate. They also found higher numbers of vernolateusing bacteria in soils treated with vernolate treatment in previous years.

EPTC-butylate cross-adaptation. In a study conducted by McCusker et al. (1988), it was reported that at three locations in South Carolina (Dothan, Varina and Wagram), evolution of 14CO2 from 14C-EPTC was greater from soils with a history of butylate applications than in corresponding soils without a history of butylate. This suggests that cross-adaption of EPTC in butylate-history soil occurred. In Varina butylate-history soil, a lag period of 8 days occurred while no obvious lag period was observed in Dothan and Wagram butylate-history soils.

Microorganisms in Dothan non-butylate history soil adapted to EPTC after approximately 8 days; Butylate-adapted microorganisms were well adapted to EPTC and degraded EPTC at nearly the same rate as butylate. This would account for the poor weed control from EPTC in a butylate-history soil. Some researchers reported that there was increased performance of EPTC by rotating herbicides without EPTC for one season, but the effect of weed control was still below that obtained by the first-time EPTC treatment (Rahman and James 1983).

Butylate, cyanazine, cycloate, alachlor rotation with EPTC. Bean et al. (1988) suggested EPTC

Butylate, cyanazine, cycloate, alachlor rotation with EPTC. Bean et al. (1988) suggested EPTC rotated with cyanazine or cycloate rather than alachlor or leaving the plots untreated. EPTC rotated with cyanazine or cycloate achieved the best weed control (from 22 to 45 days after application) among all the combinations of herbicide rotations.

However, late-season weed control with cyanazine or cycloate rotated with EPTC was less efficacious compared to the first year EPTC treatment, possibly due to soil with a EPTC history being partially adapted for degradation of EPTC, or the soil that was previously treated with EPTC while rotated with cyanazine and cycloate was very sensitive to re-enhancement, although re-enhancement may take some time to occur. Results from these studies suggested that

EPTC can be rotated successfully with cyanazine, cycloate, and to a lesser extent alachlor, but not with butylate (Bean et al. 1988).

Other Thiocarbamates Biodegradation and Cross-Adaptation between Thiocarbamates. Butylate. Obrigawitch and Tobias (1982a) reported that butylate-enhanced degradation was observed in a butylate history soil. However, the rate of butylate degradation in butylate-history soil could not be compared to the rate observed for EPTC in EPTC-enhanced degradation soil (Obrigawitch et al. 1982a).

Rotating from butylate to no herbicide to butylate improved giant foxtail (*Setaria faberi* Herrm) control compared to a 3-yr butylate treatment (Rudyanski et al. 1987). Butylate degradation was slower in soil with a 2-year butylate history followed by 1-year cycloate exposure compared to soil with a 3-year butylate exposure (Wilson 1984), however, degradation was enhanced in both of the soils.

In the study conducted at Dothan, Varina and Wagram butylate-history soils in South Carolina (McCusker et al. 1988), enhanced biodegradation of butylate was observed and had almost no lag phase in all three types of butylate-history soil. Degradation of butylate in three non-butylate history soils had an obvious lag phase of 6 days. Microorganisms in non-butylate history soil in Dothan adapted to butylate as a carbon source or N and S source as soon as butylate was applied. However, butylate degradation was slower and more linear in the Varina and Wagram soils, indicating that the microorganisms in these soils had not adapted to butylate at the beginning of application. These results showed the differences between microorganism species in different soils. Enhanced degradation of butylate occurred in these soils after repeat usage.

*Vernolate*. Tal et al. (1989b) found that in soil previously treated for 6 years with vernolate, degradation of EPTC was dramatically enhanced, and in only 9 days, 40% of <sup>14</sup>C-EPTC was degraded to <sup>14</sup>CO<sub>2</sub> completely, while 36% of <sup>14</sup>C-EPTC was lost to volatilization. On the 15th day of incubation, no organic phase of <sup>14</sup>C-EPTC was found in the soil, rendering EPTC completely useless for weed control.

Butylate-adapted microorganisms were not cross-adapted to vernolate in Varina and Wagram butylate-history soils, since the rate of vernolate degradation did not vary much between the butylate-history soils and controls sampled from these two locations (McCusker et al. 1988). Nevertheless, vernolate degradation in Dothan butylate-history soil was higher than the rate in non-butylate history soil, indicating these butylate-adapted microorganisms in Dothan soil were cross-adapted to vernolate but with less efficiency than for EPTC.

Pebulate. Also in the study of McCusker et al. (1988), pebulate degradation in Dothan butylate-history soil was greater than non-butylate-history soil. This fact indicated that cross-adaption of pebulate in butylate-history soil had occurred in the Dothan butylate-history soil. However, no rapid <sup>14</sup>CO<sub>2</sub> evolution from <sup>14</sup>C-pebulate was observed and pebulate was degraded slower than butylate or EPTC in the Dothan butylate-history soil. Evolution of <sup>14</sup>CO<sub>2</sub> from butylate-history soils in Varina and Wagram was similar to their respective non-butylate history soil, which suggested that butylate-adapted microorganisms in Wagram and Varina soils were not cross-adapted for pebulate (McCusker et al. 1988).

*Cycloate*. Cycloate degradation was not affected by previous applications of butylate in either of the three soils (McCusker et al. 1988). Evolution of <sup>14</sup>CO<sub>2</sub> was lowest among all the thiocarbamate herbicides tested, and no obvious cross-adaption of cycloate was observed in any of the three soils used in this study. This may explain the long half-life of cycloate in soil.

Cycloate contains a cyclohexyl group which apparently makes it more resistant to microbial degradation (Senseman 2007). Cycloate was also recommended by Bean et al. (1988) to rotate with EPTC in order to produce better weed control in the field.

Evolution of <sup>14</sup>CO<sub>2</sub> by microbial degradation across soil type and previous butylate history was in the following order:

(most <sup>14</sup>CO<sub>2</sub> detected) EPTC=butylate>pebulate>vernolate>cycloate (least <sup>14</sup>CO<sub>2</sub> detected)

Metam sodium. Metam sodium (Vapam) is a dithiocarbamate soil fumigant with high solubility
and volatility used to control weeds, fungi, nematodes and insects. It is used mostly as a soil
sterilizer. Although aqueous metam could keep the stability for up to 2 weeks in soil (Gray 1964),
it will be degraded very quickly to MITC, which is the active ingredient against weeds, pests and
other target organisms (Munnecke et al. 1962). It has been reported that high temperature and
low soil moisture facilitated the degradation of metam to MITC, while total time required for
degradation varied from 2-7 h (Turner and Corden 1963).

In another study (Gerstl et al. 1976), researchers found that metam sodium broke down to MITC rapidly. At a condition of soil moisture contents below saturation, half-life of metam was usually shorter than 30 min and MITC was found to persist up to 2 weeks. MITC adsorption in soil was low, so most of the MITC was found in the soil solution. Gerstl et al. reported that the breakdown of metam sodium in soils with high clay or high organic matter content was faster than in soils with a sand texture or low organic matter.

Warton and Matthiessen (2005) reported that enhanced biodegradation of the metam sodium was readily induced in a naturally acidic sandy soil (pH 4.2 measured in 0.01M CaCl<sub>2</sub> by multiple treatments), but only when the pH and calcium concentration were raised simultaneously using calcium carbonate (lime). Enhanced biodegradation was not induced when

soil pH was raised alone with magnesium carbonate, nor when calcium alone was raised by adding calcium chloride. In limed sand treated monthly for 12 months, the degradation rate increased to the point that dissipation was completed within 24 h of application after the fifth metam-sodium treatment at pH 7.8 and after the eighth metam-sodium treatment at pH 6.8. Pesticide concentration was reduced, but not eliminated, at pH 5.8 and was unchanged at pH 4.8. When metam-sodium was applied bi- and tri-monthly, the degradation rate also increased when soil pH was raised with calcium carbonate, but to a lesser extent than with monthly applications. In an acid loam soil amended to the same pH values with calcium carbonate and treated monthly, there was no correlation between soil pH or calcium concentration and degradation. The results showed the interdependence of pH and calcium concentration in enhancement of biodegradation of metam sodium, but confirmed that this phenomenon depended on interaction with soil type and frequency of application, which probably together acted to affect the community abundance, composition and activity of the soil microbes.

Duration of Enhanced Degradation. Number of years for enhanced thiocarbamate biodegradation to occur. In the study conducted by Bean et al. (1988) concerning duration of enhanced degradation of EPTC influenced by herbicide rotation, application timing and location, found that enhanced biodegradation of EPTC occurred after the second annual application of EPTC (Bean et al. 1988). Residue of EPTC significantly decreased in soil treated with EPTC in previous years than the untreated soil. However, residue of EPTC in soil treated with EPTC the previous year was significantly higher than that in soil treated with EPTC for 5 consecutive years. This fact suggested that enhanced degradation of EPTC occurs after the second annual application of EPTC, but the degradation rate is not as fast as the rate in soil treated with EPTC

for continuous 5 years. This result also indicated that the microbial ability of enhanced biodegradation of EPTC is gradually induced. Obrigawitch et al. (1982b) also confirmed that only one application of EPTC was enough to induce rapid EPTC degradation a year later. *Duration of enhanced degradation*. Field studies indicated that the ability of soil microorganisms to cause enhanced biodegradation of thiocarbamates may persist in soils for more than 1 year after last thiocarbamate application. EPTC performance was still below first-time application when alachlor and metolachlor were alternated with EPTC in the field (McCusker et al. 1990).

Duration of enhanced degradation in soil may be influenced by the chemical and physical characteristics of the soil as well as soil microbial composition and population (Menkveld and Dekker 1984). In North Dakota, oat (*Avena sativa* L.) control with EPTC in 1985 was less in plots treated with 4.5 and 9 kg ai/ha EPTC 3 years ago than in previously untreated plots. Degradation of EPTC in a field previously treated with three annual butylate applications still remained enhanced 3 years later after the last application of butylate (Menkveld and Dekker. 1984).

In a study conducted in Clay center and Scottsbluff, Nebraska (Bean et al. 1988), laboratory analysis of Scottsbluff soil samples indicated that the soil had lost its enhanced EPTC degradation capability 18 months after the second of two annual EPTC applications. In Clay center, soil was not enhanced 30 months after the second of two annual EPTC applications. The different soil texture and organic content as well as the soil pH may account for the difference of reversion time. Researchers suggested that rotation of EPTC with other non-thiocarbamate herbicides or with cycloate since it would give the soil an extra 12 months for reversion to take place.

Evaluating the persistence of EPTC by cucumber bioassay. Danielson et al. (1961) tested the persistence of thiocarbamates using different dicot and monocot plants for bioassay. Herbicides were applied either on the soil surface as pre-emergence treatments, or treated pre-plant incorporated (PPI) followed immediately by 1 inch irrigation water. Thiocarbamates used include EPTC, CDEC, CIPC, CEPC and MIPC. Bioassay plants used in this study were mustard greens, *Brassica juncea* (L.) Czern; snap beans, *Phaseolus vulgaris* L.; annual ryegrass *Lolium multiflorum* Lam.; lettuce, *Lactuca sativa* L. and cucumber, *Cucumis sativus* L. All bioassay plants were direct-seeded in single rows in each plot: at the day of treatment (in all preemergence plots); 2 weeks after treatment (in B sub-plots of PPI); 4 weeks after treatment (in C sub-plots of PPI). Results were obtained based on relative growth and visible injury ratings for each bioassay plant.

They found that EPTC performed more consistently in a extended period of 4 weeks than other thiocarbamates used in this study. In most of the cases, PPI increased EPTC persistence in soil than when applied preemergence. Annual ryegrass was the most sensitive bioassay plants in this study, 1 lb ai/A EPTC was able to cause complete kill in preemergence treatments and PPI 2-week sub-treatments. It also caused 91 % kill in PPI 4-week sub-treatments. Cucumber was the most sensitive dicot plant to EPTC of all four dicot bioassay plants used: 8 lb ai/A EPTC treatment caused complete kill in preemergence treatments; 97% kill in PPI 2-week and 82% kill in PPI 4-week sub-treatments. Lettuce was the second most sensitive dicot bioassay plant to EPTC, followed by mustard greens and snap beans. Snap beans were not very responsive to EPTC and 16 lb ai/A EPTC treatment applied preemergence; PPI 2 weeks before seeding and PPI 4 weeks before seeding caused only 16%, 37% and 57% kill, respectively. The results of their study suggested cucumber can be used as a good indicator of EPTC persistence in soil due

to its high sensitivity to EPTC. Kratky and Warren (1971) also concluded that cucumber could be used in rapid root bioassays, in which cucumbers were sensitive to most of the 42 herbicides tested in their study except for the photosynthetic inhibitors.

Genes for Degradation of the Thiocarbamate Herbicide. Muller et al. (1988) reported that the thiocarbamate degradative ability of bacterial isolates was plasmid-encoded, which indicated the possibility of transfer of the genetic material encoding for EPTC degradation in the environment. Enhanced degradation of EPTC may be caused by rapid transfer of plasmids containing EPTC-degrading gene between microbes by conjugation. This transfer was efficient from one generation to another generation. However, the detail of the transfer was still not clear.

Recently, researchers found that degradation of EPTC and atrazine was related to an indigenous plasmid in *Rhodococcus* Sp. Strain TE1 (Shao et al. 1995). Plasmid DNA libraries of *Rhodococcus* sp. Strain TE1 were built in a *Rhodococcus-Escherichia* coli shuttle vector, pBS305, and was transferred to *Rhodococcus* sp. Strain TE3, a mutant of Sp1 without the ability to degrade EPTC, to select transformants capable of growing on EPTC as the only carbon source. In this study, the results indicated that ept 1 gene, which coded for the enzyme needed for EPTC degradation, was located on a 6.2-kb Kpn1 fragment. This cloned fragment also contained the gene required for atrazine *N* dealkylation (atr A). The plasmid carrying the cloned fragment could be incorporated into some other *Rhodococcus* strains. Subcloning of the 6.2-kb fragment to distinguish between EPTC-and atrazine-degrading genes failed.

**Pathways of EPTC Degradation in Microorganisms.** In one study concerning fate of <sup>14</sup>C-EPTC in a soil showing enhanced degradation problem, results of thin-layer chromatography of the organic phase clearly showed that most radioactivity co-chromatographed with the original <sup>14</sup>C-EPTC (94-97%); two metabolites of EPTC degradation, the sulfoxide and the sulfone,

comprised only a small part (1%-3% of total <sup>14</sup>C) of the fraction (Tal et al. 1989b). Although the radioactivity of the organic phase changed in this experiment, the distribution of these three compounds remained constant. No data showed that these EPTC metabolites accumulated in the soil during the degradation process. This was consistent with the results reported by Casida et al. (1974) who had shown the metabolism of thiocarbamates in soil occurred via oxidation to unstable sulfoxides which were further mineralized. Wilson and Rodebush (1987) hypothesized that the main pathway of degradation of EPTC in EPTC history soil is hydroxylation, while in non-EPTC history soil, it is the sulfoxidation process.

At least three possible initial reactions by which isolated strain JE1 degrades EPTC were proposed by Ankumah et al. (1995):

- The first reaction involves oxidation of the S atom to form sulfoxide. This compound
  will be further oxidized to the sulfone. Further metabolism of these oxidized
  compounds is not confirmed.
- 2. EPTC is first hydroxylated at the *a*-propyl C, which results in the formation of *a*-hydroxypropyl EPTC. This compound is unstable and breaks down to *N*-depropyl EPTC and propionaldehyde. *N*-depropyl EPTC is hydrolyzed to *s*-ethyl carbonothioic acid and propylamine. The *s*-ethyl carbonothioic acid is then demethylated to *s*-methyl carbonothioic acid. This product is hypothesized to further degrade to form CO<sub>2</sub> and methyl mercaptan.
- 3. EPTC is hydroxylated at the *a*-ethyl C, which leads to the formation of acetaldehyde, dipropylamine and carbonyl sulfide.

Ankumah et.al. (1995) tested the amount of different metabolites of <sup>14</sup>C-EPTC using gas chromatography-mass spectrometry analyses. The largest amount of the *N*-depropyl EPTC

produced among all the metabolites was detected throughout the incubation period, and this suggested hydroxylation of the *a*-propyl C of the *N*, *N*-dialkyl moiety may be the major pathway in the microbial metabolism of EPTC. It may also explain why dietholate, which is believed to inhibit the hydroxylation pathway, slowed down the degradation of EPTC in soils exhibiting enhanced degradation (Obrigawitch and Tobias 1982a; Roeth. 1986). Nevertheless, dietholate did not have any effect on the EPTC degradation in soils with no EPTC treatment history. This fact suggested that the hydroxylation process might be induced by repeat usage of EPTC (Ankumah et al. 1995). This also supported the hypothesis of Wilson and Rodebush (1987) that main pathway of EPTC in EPTC history soil is hydroxylation, while in none EPTC history soil, sulfoxidation was considered to be the dominant one. Thus, availability of different pathways for the microbial degradation of EPTC in soil may explain the very efficient degradation of EPTC in soil with repeat applications of this herbicide.

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## Introduction

Bermudagrass (*Cynodon* spp.) infestation is a serious problem in warm-season turfgrass management and sod production in the Southeast due to its fast-growing and fast injury-recovering habit. If the sod planting site is contaminated by bermudagrass before or during planting, it will be very costly and laborious to eradicate bermudagrass in established turf which usually takes years as well as multiple postemergence herbicide treatments to correct this problem (Griffin et al. 1994; Boyd 1991). In some extreme cases, it may require complete renovation and replanting of the infested turf.

To prevent this weed problem, many states require pre-planting fumigation and sod examination before sod can be labeled as certified sod. However, due to the toxic and volatile nature of most liquid fumigants, pre-planting fumigation can be both expensive and hazardous. Equipment limitations, toxicity issues, potential ground water contamination, Personal protective equipment (PPE) requirements and high summer temperature of the Southeast occasionally render this practice nearly impossible (Unruh et al. 2002).

In the past years, the fumigant methyl bromide was widely used as an effective tool to control bermudagrass before turfgrass planting. However, its registration and usage in United States was officially phased out by EPA on Jan. 1, 2005 due to ozone depletion. Many research studies have been conducted so far to find possible alternatives for methyl bromide for various agronomic and horticultural crops (Duniway et al. 1999; Gilreath et al. 2004, Gilreath and Santos 2005). It has been reported that several fumigants or fumigant combinations may have the

potential to be used as methyl bromide alternatives in weed control. Nevertheless, very few performed equally well as methyl bromide, especially in bermudagrass control and problems stated above about fumigation process still exist (Unruh et al. 2002; Noling and Becker 1994).

Walker et al. (2008) found that thiocarbamate herbicide EPTC was an effective and economical method to control bermudagrass. EPTC provided complete control of both hybrid and common bermudagrass at two locations (Tuskegee and Tallassee) in Alabama. Yellow nutsedge control averaged 94% at Tallassee location. In 2008, their study results showed that EPTC suppressed bermudagrass more than any rate of dazomet treatments (100, 200, 300, 400 lb ai/A) used. EPTC at 7 lb ai/A + dazomet at 400 lb ai/A provided best bermudagrass control of all chemical treatments but difference within EPTC at 7 lb ai/A and EPTC at 7 lb ai/A + dazomet at 400 lb ai/A was not significant.

EPTC has been confirmed to be an effective herbicide for bermudagrass control.

However, repeat usage of EPTC will cause enhanced biological degradation problems, which reduces EPTC soil retention and decreases its efficacy. Previous literature suggested that addition of the fumigant dazomet may correct this problem by injuring the microorganisms that are responsible for degrading EPTC in soil (Tal et al. 1989), but little information is known concerning possible injury to warm-season turfgrass by residue of EPTC and EPTC + dazomet combination after turfgrass is planted back. Beste and Schreiber (1970) found that 2,4-D can impart safening to corn and soybean against EPTC injury. Therefore, the objective of this study was to evaluate EPTC as a preplant herbicide in warm-season sod production; investigate minimal turfgrass plant-back interval after EPTC and EPTC + dazomet treatment without incurring significant turfgrass injury and growth decrease; also, to evaluate 2,4-D as a potential safener to three warm-season turfgrasses against EPTC injury.

## **Materials and Methods**

Auburn University Turfgrass Research Unit Plant-Back Study. Field research was conducted at the Auburn University Turfgrass Research Unit (AUTRU), in Auburn Alabama during 2008-2010 to determine optimum plant-back interval for zoysiagrass (Zoysia japonica Steud.) and St. Augustinegrass [Stenotaphrum secundatum (Walter) Kuntze] following soil application of EPTC and EPTC + dazomet. Oxadiazon was applied as a reference herbicide. Soil at the AUTRU was identified as a fine loamy, siliceous, thermic Plinthic Paleudults with medium levels of available P, Mg, K, and Ca and soil pH was determined to be 5.7. After grass planting, a scheduled monthly maintenance fertilizer program using triple 13 or 30-3-10 was applied to maintain proper soil fertility. Treatments, pesticide formulations, rates used and herbicide application information for this experiment are listed in Table 1. Experiment design was a split block design with four replications. Herbicide plot size was 1.4 by 6.1 m and plant-back plot size was 1.4 by 1 m within herbicide plots and there were appropriate buffers between blocks and plant-back plots. All 10 herbicide treatments were applied randomly in each block in one direction and all three plant-back intervals were randomly assigned in each block in a direction vertical to the previous one. EPTC had to be soil incorporated immediately after spraying due to its high volatility. Because the power tiller used for soil incorporation required at least 1.5 m for starting and stopping, it was necessary to apply EPTC and EPTC + dazomet in strips because incorporation could not be done correctly in small plots. Soil incorporation was done with a 1.5-m power tiller operated to a soil depth of 10 cm. It has been shown (Ross and Lambi 1985) that a power tiller

incorporates herbicides to the depth of cut. Oxadiazon was sprayed on the soil surface and was not mechanically incorporated. After all herbicide application, 6.4 mm of sprinkler irrigation was applied to the experimental area to activate oxadiazon and to seal the soil surface to reduce potential for volatilization of EPTC. Dazomet was applied 1 to 2 days after the EPTC using a drop-type granular spreader. It was then soil incorporated the same as the EPTC and again sprinkler irrigated the same as described above.

Specific turfgrass cultivars utilized in the experiment included 'BK-7' zoysiagrass and 'Palmetto' St. Augustinegrass. For each species, stolons with three to five nodes for the two turfgrasses were planted in plastic cones (2.54 cm diameter, 11.4 cm length) in a greenhouse environment 2 months before field planting. Plant-back plots included five rooted stolons from the cone containers that were planted in a star-shaped pattern with a spacing of 25 cm among the five.

2008. All treatment applications were completed on 30 July and turfgrass from the cones was planted on 7 August, 18 August and 5 September, representing 1, 3 and 5 week plant-back intervals, respectively. After about 10 month, stolons and rhizomes of each plant-back plot was harvested on 20 May, 2009 using a shovel. Soil was removed by vigorous shaking, and collected plants were placed in paper bags and dried for 4 days at 60 C. After drying, samples were again shaken to remove any remaining soil and dry weight grams were determined.

2009. Same treatments were applied again to the 2008 plots representing 2 years of treatment and another adjacent experiment received the same treatments for the first time. Oxadiazon and EPTC were treated on 5 August, 2009 but dazomet treatments were not applied until 7 August due to a thunderstorm on the afternoon of 5 August. It was determined that there was no need to wait 3 or 5 weeks before planting back turfgrass based on the results of 2008 plant-back study.

Therefore, plant-back interval was shortened and turfgrass plantings were made on 14 August, 21 August and 28 August representing plant-back intervals of 1, 2 and 3 weeks. Five rooted stolons of zoysiagrass and St. Augustinegrass from cone containers were planted at each planting date in each 2009 experiment. Cucumbers (bioassay) were also planted in each herbicide plot following the same turfgrass plant-back schedule. Six seeds were planted in three hills per plot 1, 2 and 3 weeks after EPTC treatment and allowed to grow 4 weeks before harvesting. The cultivar was 'Straight Eight' obtained from Ferry-Morse Seed Co. Fluton, KY, USA. Cucumber plants were harvested with a small shovel and plants were shaken vigorously before placing in paper bags. Bagged plants were oven-dried at 60 C for 4 days then dry weight was measured and recorded. Initial data collection of turfgrass planted for the 2009 experiments began on 20 January, 2010 where length of the three longest stolons in each plant-back plot was recorded. All plots were harvested for dry weights beginning 25 May, 2010. Harvesting and drying was done the same as in the 2008 experiment.

Generalized linear mixed model procedure (PROC GLIMMIX) of SAS® program was used to perform data analysis in this study. Grass dry weight and average stolon length of the three longest stolon were analyzed in a model containing fixed effects of treatment, plant-back interval and treatment  $\times$  plant-back interval and random effects of block, block  $\times$  treatment, block  $\times$  plant-back interval. Fixed effects were tested for significance by Type III Tests of Fixed Effects. If fixed effects treatment  $\times$  plant-back interval and plant-back interval were confirmed to be significant at  $\alpha = 0.05$  level, treatment means in each plant-back interval or plant-back means in each treatment would be further separated by Least Significance Difference (LSD) at P $\leq$ 0.05. 2010. A quantitative analysis of EPTC using GC-MS was conducted in 2010 with the objective of determining EPTC soil retention under field condition. No turfgrass was planted back that

year in the field. Soils at the AUTRU which received EPTC treatment in both 2008 and 2009 or an single application in 2009, which represented 2 or 1 years of EPTC treatment history, respectively. EPTC, 7.84 kg ai/ha, application was made on 23 July, 2010 to all 2 and 1 years EPTC history plots, followed by immediate soil incorporation and 6 mm water seal as previously described. Soil was sampled from these plots at 1, 3, 7, 10, 14, 18 and 21 days after EPTC treatment. Since all EPTC treated plots were rototilled during herbicide incorporation and received 2 mm daily irrigation after treatment, the topsoil was loose and moist in each plot. It was impossible to utilize a soil sampler or a soil auger to sample soil, therefore the soil was sampled from the soil surface to a depth of 7.6 cm by hand and small handhold-shovel in order to maintain sampling accuracy. Ten samples were randomly sampled in each herbicide plot and then thoroughly mixed and screened though a 2-mm sieve before oven-drying two days at 60 C to measure soil moisture content. Twenty grams on a dry-weight equivalent of wet soil from each plot was taken for EPTC extraction. Each soil sample was placed in 200 ml conical flask and 100 ml of ethyl acetate was added and placed on a wrist-action shaker. (Model 75, Burrell Scientific. Pittsburgh, PA. USA.) and shaken for 1h after which the supernatant liquid was decanted from the sample. This process was repeated twice for a total of three extractions. All the supernatant and slurry mixture collected was filtrated through Whatman number 40 filter paper and 20 ml ethyl acetate was used to flush the filter paper after filtration. Then, the filtered liquid was evaporated using a rotatory evaporator at 40 C water-bath temperature to a final volume of 10 ml. Extraction efficiency was confirmed to be over 90% using the method described above in AUTRU soil quantitatively incorporated with 7 ppm EPTC.

Mass Spectrometry-Gas chromatography was performed at Department of Biochemistry, Auburn University, Auburn AL, to monitor field EPTC decomposition rate. An Agilent Technology 6890N GC (Agilent Technologies, Inc. Santa Clara CA 95051) equipped with an Agilent J&W scientific DB-5 column (I.D., 0.25 mm, Length 15m, Film Thickness 0.25 µm, Agilent No. 122-5012) was used in this study. Injector temperature was kept at 250 C and 2 µl injection volume was injected splitless. Carrier He gas flow was set at 2.9 ml/min. The following temperature program was used for all the samples in this study: temperature was set at 100 C initially for 3 mins, then increased 8 C per minute until a final temperature of 280 C. With these settings, EPTC had a retention time of 9.4 min and the integral area of major fragmentation peak of EPTC was 128.108. EPTC residual amount of soil sample was obtained by comparing the sample value to a standard curve made from known concentrations of EPTC solutions. Gulf Coast Research and Extension Center 2,4-D Safening Study. A field study was conducted at the Gulf Coast Research and Extension Center (GCREC), Fairhope, AL in 2009 (30°32′ N, 87°52′ W) to determine if 2,4-D could be used to safen vegetatively planted turfgrasses to preplant-applied EPTC and EPTC + dazomet. Previous research has shown safening to corn and soybean with 2,4-D application to soil and seed treatment solution (Beste and Schreiber, 1970). Soil type at the GCREC was a Malbis sandy loam, fine-loamy, kaolinitic, thermic typic Kandiudults. Specifics for herbicide treatments, formulations, use rates and application dates used in this study are shown in Table 2. Experimental design was a split block design with four replications. Treatments were randomly assigned in each block in one direction and three plant-back intervals were randomly assigned within each herbicide plots in the direction vertical to the previous one. Block size was set as  $15 \times 30$  m and plant-back plot size was  $1.7 \times 2.5$  m. It was necessary to apply EPTC and dazomet in strips since the vertical-action tiller requires at least 1.5 m outside each plot to start and stop and thus traditional smaller plots could not be used. Dazomet was applied on 24 June, 2009 with a drop-type granular spreader and was immediately soil incorporated with the vertical-action tiller cutting to a depth of 10 cm. Oxadiazon was applied to the soil surface and EPTC was applied and soil incorporated as previously described, both on 1 July, 2009. Six mm of irrigation water was used to seal the soil surface to prevent EPTC volatilization and to activate oxadiazon. One week after EPTC and oxadiazon application, 2,4-D was applied on 8 July to the soil surface and turfgrass planting was conducted 1, 2, and 3 weeks later. Turfgrass cultivars used in this study include Tifway bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy], BK-7 zoysiagrass and Palmetto St. Augustinegrass. Each was planted using a single 10-cm-diameter sod plug. The three planting dates were 15 July, 22 July and 29 July, 2009, which represented 1, 2, and 3 week after the 2,4-D application.

Stolon length was measured on 12 August, 19 August and 26 August, 2009. All stolons longer than 2.54 cm on each sod plug were measured and recorded. Turfgrass ground coverage was obtained by analyzing pictures with image software. Before taking pictures, all experimental plots received careful hoeing and hand-weeding in order to ensure that no increase in measurements of ground vegetation coverage was caused by weeds. Then, pictures of each sod plug were taken on 8 October, 2009 using a Canon Powershot G9 camera (Canon USA., Inc. Lake Success, NY. USA) mounted on top of a portable light box (61 cm long, 51 cm wide and 56 cm high). Four fluorescent light bulbs were mounted on the upper inside which supplied uniform artificial lighting for each photograph. Pictures of each sod plug were analyzed using imaging software SigmaScan Pro 5 (Systat software, Inc. Chicago, IL. USA) which allowed determination of percent of turfgrass ground coverage by calculating the ratio of all the pixels that fell into the previously set color threshold to total picture pixels as described by Richardson et al. (2001). Color threshold setting (Hue and Saturation) for bermudagrass, St. Augustinegrass

and zoysiagrass were: Hue 32-114, Saturation 20-100; Hue 33-114, Saturation 20-100; Hue 34-114, Saturation 20-100, respectively. Total picture pixels were set as 307,200 (640×480). Other camera settings were ISO 100, f/3.2; focal length 7 mm; exposure time 1/30 s; auto white balance (AWB).

Generalized linear mixed model procedure (PROC GLIMMIX) of SAS® program was used to perform data analysis in this study. Percent of turfgrass ground coverage and average stolon length were analyzed in a model containing fixed effects of treatment, plant-back interval, treatment  $\times$  plant-back interval and random effects of block, block  $\times$  treatment, block  $\times$  plant-back interval. Fixed effects were tested for significance by Type III Tests of Fixed Effects. If fixed effects interaction and plant-back interval were confirmed to be significant at  $\alpha$ =0.05 level, treatment means in each plant-back interval or plant-back means in each treatment would be further separated by Least Significance Difference (LSD) at P≤0.05.

## **Results and Discussion**

Auburn University Turfgrass Unit Plant-Back Study. St. Augustinegrass 2008. Dry weight data for Palmetto St. Augustinegrass planted in 2008 and harvested in 2009 showed there were significant dry weight differences among treatments within the plant-back interval of 1 week after treatment (1 WAT). At this earliest plant-back interval, dazomet at 336 and 448 kg ai/ha produced the highest St. Augustinegrass dry matter (Table 3). Lowest dry matter values were recorded with EPTC + 224 and 336 kg ai/ha of dazomet. All other treatments produced intermediate levels of dry weight. No significant differences were found within EPTC and four EPTC + dazomet treatments. St. Augustinegrass dry weight data for the 3 WAT plant-back interval showed no differences among treatments. Delaying St. Augustinegrass plant-back interval to 5 WAT produced some differences among treatments. EPTC + dazomet at 448 kg ai/ha produced higher St. Augustinegrass dry weight than the same chemical combination that included the two lower rates of dazomet. Plant-back interval effects within chemical treatments showed no plant-back differences within all treatments containing EPTC + dazomet or dazomet alone at 112 kg ai/A. The two higher rates of dazomet showed significantly lower dry matter for the two later plant-back intervals compared to the 1 WAT plant-back interval. These same effects were evident for the oxadiazon treatment (Table 3). EPTC alone or with dazomet could be safely used on newly planted St. Augustinegrass when the plant-back interval was at least 3 WAT. Delaying plant-back to 5 WAT did not adversely affect St. Augustinegrass dry weights in treatment EPTC and EPTC + dazomet while delaying plant-back following dazomet or oxadiazon treatment did adversely affect dry weight production.

St Augustinegrass 2009. With one exception, average stolon length taken from the longest three stolons showed all chemical treatments produced stolons that were statistically equivalent in length for the first two plant-back intervals. However, significantly shorter stolon length was evident for these treatments when the plant-back interval was increased to 3 WAT. The exception was dazomet at 448 kg ai/ha which produced shorter stolon length as plant-back interval increased in time (Table 4).

St. Augustinegrass dry weight was significantly affected by soil chemical treatment when the plant-back interval was 1 WAT. All rates of dazomet produced higher dry weights except the 112 kg ai/ha rate. All treatments containing EPTC alone or in combination with dazomet produced dry weight equivalent to oxadiazon in 1 WAT plant-back interval. There were no differences in dry weight production among any soil chemical treatments when the plant-back interval was 2 or 3 WAT (Table 5). When examining dry weight production affected by plant-back intervals within a soil chemical treatment, all dazomet alone treatments produced lower dry weight when the plant-back interval was 2 and 3 WAT. Conversely, EPTC + dazomet at the three higher rates produced St. Augustinegrass dry weight that was equivalent for all three plant-back intervals. The remaining treatments of oxadiazon, EPTC alone, and EPTC + dazomet at 112 kg ai/ha generally produced equivalent dry weight for the first two plant-back intervals and lower dry weight for the 3 WAT interval (Table 5). Planting of St. Augustinegrass 1 week after EPTC or EPTC + dazomet soil treatment produced St. Augustinegrass growth that was equivalent to oxadiazon but generally less than dazomet applied alone, particularly at the higher dazomet rates.

Zoysiagrass 2008. Dry weight of BK-7 zoysiagrass was affected by chemical treatments in the first two plant-back intervals. Clearly the dazomet 448 kg ai/ha treatment produced the highest zoysiagrass dry weight in 1 WAT plant-back interval. Statistically equivalent chemical treatments were oxadiazon, EPTC + dazomet at 448 kg ai/ha and dazomet at 224 kg ai/ha. EPTC alone produced the lowest dry weight in the 1 WAT plant-back interval. Very similar results were observed among chemical treatments when the plant-back interval was 3 WAT; higher rates of dazomet alone produced highest dry weight and EPTC alone produced the lowest. Conversely, no differences were evident among chemical treatments when zoysiagrass planting was delayed to 5 WAT (Table 6). Plant-back interval within a chemical treatment generally showed a decline in dry weight as interval between chemical application and zoysiagrass planting increased but there were three exceptions where plant-back interval did not produce significant differences in dry weight: 1) EPTC alone; 2) EPTC + dazomet at 224 kg ai/ha; 3) EPTC + dazomet at 448 kg ai/ha. Within all other chemical treatments, zoysiagrass dry weight was equal for the 1 and 3 WAT plantings with lower dry weight for the 5 WAT plant-back interval (Table 6). These data show that it was reasonably safe to plant zoysiagrass at any of the three plant-back intervals.

Zoysiagrass stolon length from the 2009 experiment showed two major groupings: 1) three treatments had equivalent stolon length for the 1 and 2 WAT plant-back interval with shorter stolon length for 3 WAT (EPTC, oxadiazon and the highest rate of dazomet; 2) six treatments consisting mainly of EPTC + dazomet and the two lower rates of dazomet alone produced average stolon length that was highest for the 1 WAT interval and shorter but statistically equivalent stolon length for the 2 and 3 WAT plant-back intervals (Table 7). No significant differences were found among three plant-back intervals in dazomet at 336 kg ai/ha.

There were two major groupings for zoysiagrass dry weight for the 2009 experiment: 1) Five chemical treatments where dry weight was higher for the 1 WAT and lower but statistically equal dry weight for 2 and 3 WAT plant-back intervals. These treatments consisted of the three EPTC + dazomet treatments and the two lower rates of dazomet; 2) The remaining four treatments generally produced lower dry weight for the 3 WAT plant-back interval compared to 1 and 2 WAT plant-back interval (Table 8).

Cucumber bioassay 2009. When cucumber were seeded 1 WAT, dazomet treatments produced the highest dry weights. Significantly lower dry weights were evident with EPTC alone or EPTC + dazomet while no dry matter was produced in the oxadiazon-treated plots. Addition of dazomet at various rates with EPTC did not produce significant lower cucumber dry weight than EPTC alone. In 2 WAT, cucumber dry weight was equivalent among the majority of treatments with two exceptions, which showed oxadiazon alone produced the lowest dry weight and the highest rate of dazomet produced the highest dry weight. No significant differences were evident among any chemical treatments when seeding was done 3 WAT (Table 9). There were two major groupings when plant-back intervals were evaluated within chemical treatments. Cucumber dry weight generally declined as plant-back interval increased with the dazomet treatments; particularly from the 1 WAT to 3 WAT. The second grouping consisted of the remaining oxadiazon and EPTC treatments which produced no effects on dry weight production over the three plant-back intervals (Table 9).

Gulf Coast Research and Extension Center 2,4-D Safening Study. Beste and Schreiber (1970) showed that 2,4-D could be used to protect corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] from EPTC injury. Therefore, research was initiated to determine if 2,4-D could be used to safen three warm-season turfgrasses from EPTC injury.

Bermudagrass. Average stolon length (Table 10), total stolon length (Table 11) and percent ground cover (Table 12) data showed 2,4-D did not increase tolerance of Tifway bermudagrass to EPTC when compared to the other chemical treatments or across the three plant-back intervals. Average stolon length was reduced approximately 50% for treatments containing EPTC compared to those not receiving EPTC for the 1 WALT and 2 WALT plant-back intervals. Only minor differences were evident among chemical treatments at the 3 WALT plant-back interval (Table 10). These three sets of data showed that Tifway bermudagrass could be planted as early as 1 WALT or 2 weeks after EPTC application if some slowed growth could be tolerated. However, no adverse effects of EPTC were evident to Tifway bermudagrass from EPTC when the plant-back interval was 3 WALT or 4 weeks after EPTC application. Addition of dazomet did not increase EPTC toxicity to bermudagrass.

Zoysiagrass. Average stolon length for BK-7 zoysiagrass ranged from 4 to 5 cm and was not affected by plant back intervals or by chemical treatments (Table 13). However, total stolon length data showed differences in both plant-back intervals and chemical treatments (Table 14). Dazomet followed by (fb) EPTC and dazomet fb EPTC fb 2,4-D produced significantly higher total stolon length when the plant-back interval was 1 WALT. It could not be determined if this response was due to the nematacidal/fungicidal properties of dazomet or potential safening from the 2,4-D. The remaining two plant-back intervals, 2 WALT and 3 WALT, showed oxadiazon and 2,4-D generally produced less total stolon length. This response may have been due to oxadiazon having a much longer soil residual than EPTC (Herbicide handbook, 2007) and it was difficult to keep the 2,4-D-treated plots free of weeds, particularly grass species. Within chemical treatments, plant-back intervals affected only EPTC and EPTC fb 2,4-D. Both treatments responded the same and produced significantly less total stolon length when the plant-back

interval was 1 or 2 WALT. From this, it can be concluded that 2,4-D did not safen EPTC on BK-7 zoysiagrass. No significant differences were evident among the three plant-back intervals or the seven chemical treatments in percent zoysiagrass ground cover as determined by photograph analysis (Table 15).

St. Augustinegrass. Average stolon length of Palmetto St. Augustinegrass was not affected by plant-back intervals or chemical treatments. Average stolon length ranged from 9 to 18 cm over this data set (Table 16). Total stolon length was not affected by chemical treatments within a plant-back interval but there was a trend for higher numbers with the three treatments containing dazomet in the 1 WALT and 2 WALT plant-back intervals. Differences among plant-back intervals within chemical treatments were somewhat random and these differences generally showed higher total stolon lengths when the plant-back interval was 2 WALT (Table 17). Percent ground cover showed no chemical treatment effects within a plant-back interval. However, a few differences were evident within chemical treatments over plant-back intervals. For example, percent ground cover was generally higher with the shorter plant-back intervals with EPTC, dazomet fb EPTC and dazomet fb 2,4-D (Table 18). These data showed it was generally safe to plant Palmetto St. Augustinegrass as soon as 1 WALT.

Quantitatively Analysis of Field EPTC Degradation. EPTC soil residue was determined by sampling soil treated with EPTC or EPTC plus dazomet. These treatments were made to locations that had either 1 or 2 years history of EPTC use. Samples were extracted and residue analysis was done using GC-MS technology. When sampled 1 day after treatment (DAT) the highest concentration of EPTC was found in the EPTC treatment applied to soil with a 2-year history of use (Table 19). Both treatments containing dazomet generally showed lower concentrations over the 1 DAT and 2 DAT sampling dates. Since dazomet and EPTC belong to

the same chemical family, these data may indicate enhanced biological degradation of thiocarbamates and cross-adaption with the combination have occurred. Samples taken 7 DAT showed very low EPTC concentrations for all four chemical. The data showed no evidence of enhanced biological degradation with soil that had been treated 2 years with EPTC since 2 years is a relative short time of use.

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**Tables** 

Table 1. AU turfgrass research unit plant-back study treatments.

Chemical			Application
Treatment	Formulation <sup>a</sup>	Rate	Stage <sup>b</sup>
		kg ai/ha	
Oxadiazon	50 WP	3.36	PRE
EPTC	7 EC	7.84	PPI
EPTC + Dazomet	7 EC	7.84	PPI
	100 GR	112	PPI
EPTC + Dazomet	7 EC	7.84	PPI
	100 GR	224	PPI
EPTC + Dazomet	7 EC	7.84	PPI
	100 GR	336	PPI
EPTC + Dazomet	7 EC	7.84	PPI
	100 GR	448	PPI
Dazomet	100 GR	112	PPI
Dazomet	100 GR	224	PPI
Dazomet	100 GR	336	PPI
Dazomet	100 GR	448	PPI

<sup>&</sup>lt;sup>a</sup> 50 WP, 50% active by weight wettable powder; 7 EC, 7 lb ai per gallon emulsifiable concentrate; 100 GR, 100% active by weight granule.

<sup>&</sup>lt;sup>b</sup> PPI, Pre-Plant Incorporated. Herbicide was applied to the soil surface and immediately followed by mechanical incorporation; PRE, Pre-emergence. Herbicide was applied to the soil surface without mechanical incorporation.

Table 2. Gulf coast research and extension center 2,4-D safening study treatments.

Chemical			Application	Application
Treatment <sup>a</sup>	Formulation <sup>b</sup>	Rate	Stage <sup>c</sup>	Date
		kg ai/ha		
Oxadiazon	50 WP	3.36	PRE	Jul. 1
EPTC	7 EC	7.84	PPI	Jul. 1
EPTC fb 2,4-D	7 EC	7.84	PPI	Jul. 1
	3.8 SL	2.24	PRE	Jul. 8
Dazomet fb EPTC	100 GR	224	PPI	Jun. 24
	7 EC	7.84	PPI	Jul. 1
Dazomet fb EPTC fb 2,4-D	100 GR	224	PPI	Jun. 24
	7 EC	7.84	PPI	Jul. 1
	3.8 SL	2.24	PRE	Jul. 8
Dazomet fb 2,4-D	100 GR	224	PRI	Jun. 24
	3.8 SL	2.24	PPE	Jul. 8
2,4-D	3.8 SL	2.24	PRE	Jul. 8

<sup>&</sup>lt;sup>a</sup> Abbreviation: fb, followed by.

<sup>&</sup>lt;sup>b</sup> 50 WP, 50% active by weight wettable powder; 7 EC, 7 lb ai per gallon emulsifiable concentrate; 100 GR, 100% active by weight granule; 3.8 SL, 3.8 lb ai/gallon soluble liquid.

<sup>&</sup>lt;sup>c</sup> PPI, Pre-Plant Incorporated. Herbicide was applied to the soil surface and immediately followed by mechanical incorporation; PRE, Pre-emergence. Herbicide was applied to the soil surface without mechanical incorporation.

Table 3. Palmetto St. Augustinegrass dry weight as affected by soil chemical treatments and plant-back intervals, 2008. AU Turfgrass Research Unit. a,b

		Plant-back interval						
Chemical								
Treatment	Rate	1 W	AT <sup>c</sup>	3 V	VAT	5 W	5 WAT	
	kg ai/ha			g dr	y wt/plot	d		
Oxadiazon	3.36	164.7 b	A	137.9 a	AB	100.7 ab	В	
EPTC	7.84	135.2 bc	A	113.1 a	A	93.1 ab	A	
EPTC + Dazomet	7.84 + 112	113.8 bc	A	117.1 a	A	81.2 b	A	
EPTC + Dazomet	7.84 + 224	94.0 c	A	108.1 a	A	79.6 b	A	
EPTC + Dazomet	7.84 + 336	75.9 c	A	116.7 a	A	119.3 ab	A	
EPTC + Dazomet	7.84 + 448	136.0 bc	A	125.9 a	A	149.9 a	A	
Dazomet	112	163.9 b	A	141.6 a	A	131.7 ab	A	
Dazomet	224	159.9 b	A	111.6 a	AB	91.0 ab	В	
Dazomet	336	233.9 a	A	145.9 a	В	108.1 ab	В	
Dazomet	448	250.0 a	A	134.5 a	В	108.7 ab	В	

<sup>&</sup>lt;sup>a</sup> St. Augustinegrass was planted on 7, 18 August and 5 September, 2008 and harvested on 20

May, 2009; oven dried at 60 C for 4 days before measuring dry weight.

<sup>&</sup>lt;sup>b</sup> Means followed by same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviation: WAT, Weeks After Treatment.

<sup>&</sup>lt;sup>d</sup> Five rooted plants per plot.

Table 4. Average stolon length of Palmetto St. Augustinegrass as affected by three plant-back intervals within soil chemical treatment in 2009. AU Turfgrass Research Unit. a,b

Chemical			Plant-back interval	
Treatment	Rate 1 WAT <sup>c</sup>		2 WAT	3 WAT
	kg ai/ha		cm	
Oxadiazon	3.36	162 A	144 A	26 B
EPTC	7.84	157 A	142 A	30 B
EPTC + Dazomet	7.84 + 112	133 A	131 A	23 B
EPTC + Dazomet	7.84 + 224	146 A	127 A	28 B
EPTC + Dazomet	7.84 + 336	147 A	116 A	51 B
EPTC + Dazomet	7.84 + 448	121 A	139 A	67 B
Dazomet	112	169 A	134 A	39 B
Dazomet	224	169 A	141 A	29 B
Dazomet	336	175 A	139 A	50 B
Dazomet	448	180 A	128 B	69 C

<sup>&</sup>lt;sup>a</sup> St. Augustinegrass planting dates were 14, 21 and 28, August, 2009. Three longest stolons were measured on 20 January, 2010. Since fixed effect treatment and treatment × plant-back interval failed to be significant at 5% level, no treatment comparisons were performed within each plant-back interval.

<sup>&</sup>lt;sup>b</sup> Means followed by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviation: WAT, Weeks After Treatment.

Table 5. Palmetto St. Augustinegrass dry weight as affected by soil chemical treatments and plant-back intervals, 2009. AU Turfgrass Research Unit. a,b

		Plant-back interval						
Chemical								
Treatment	Rate	1 W	'AT <sup>c</sup>	2 V	VAT	3 V	WAT	
	kg ai/ha			g dr	y wt/plo	$t^d$ ————————————————————————————————————		
Oxadiazon	3.36	57.4 c	AB	94.7 a	A	23.3 a	В	
EPTC	7.84	107.6 bc	A	64.4 a	AB	38.3 a	В	
EPTC + Dazomet	7.84 + 112	98.2 c	A	52.3 a	AB	20.2 a	В	
EPTC + Dazomet	7.84 + 224	106.2 bc	A	105.0 a	A	42.2 a	A	
EPTC + Dazomet	7.84 + 336	95.8 c	A	83.4 a	A	29.8 a	A	
EPTC + Dazomet	7.84 + 448	68.7 c	A	91.1 a	A	98.2 a	A	
Dazomet	112	163.8 ab	A	74.7 a	В	32.4 a	В	
Dazomet	224	206.3 a	A	105.4 a	В	30.4 a	В	
Dazomet	336	191.1 a	A	104.0 a	В	42.4 a	В	
Dazomet	448	217.3 a	A	79.7 a	В	56.1 a	В	

<sup>&</sup>lt;sup>a</sup> St. Augustinegrass planting dates were 14, 21 and 28, August, 2009; harvested on 25 May, 2010; oven dried at 60 C for 4 days before measuring dry weight.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviation: WAT, Weeks After Treatment.

<sup>&</sup>lt;sup>d</sup> Five rooted plants per plot.

Table 6. BK-7 zoysiagrass dry weight as affected by soil chemical treatments and plant-back intervals, 2008. AU Turfgrass Research Unit.<sup>a,b</sup>

Chaminal				Plant-back in	nterval		
Chemical Treatment	Rate	1 WA	$T^{c}$	3 WA	Т	5 W.	AT
	kg ai/ha			g dry v	vt/plot <sup>d</sup>		
Oxadiazon	3.36	111.0 ab	A	116.9 a	A	68.2 a	В
EPTC	7.84	68.2 c	A	59.7 d	A	61.5 a	A
EPTC + Dazomet	7.84 + 112	98.3 bc	A	66.8 cd	AB	53.0 a	В
EPTC + Dazomet	7.84 + 224	89.6 bc	A	73.4 bcd	A	59.7 a	A
EPTC + Dazomet	7.84 + 336	92.8 bc	AB	113.8 ab	A	64.9 a	В
EPTC + Dazomet	7.84 + 448	99.3 abc	A	87.9 abcd	A	83.3 a	A
Dazomet	112	95.5 bc	A	62.0 d	AB	49.7 a	В
Dazomet	224	107.8 ab	A	69.7 cd	AB	52.1 a	В
Dazomet	336	90.6 bc	A	116.6 a	A	43.5 a	В
Dazomet	448	140.0 a	A	106.6 abc	AB	86.2 a	В

<sup>&</sup>lt;sup>a</sup> Zoysiagrass planting dates were 7, 18 August and 5 September, 2008; harvested on 20 May, 2009; oven dried at 60 C for 4 days before measuring dry weight.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviation: WAT, Weeks After Treatment.

<sup>&</sup>lt;sup>d</sup> Five rooted plants per plot.

Table 7. Average stolon length of BK-7 zoysiagrass as affected by three plant-back intervals within soil chemical treatment, 2009. AU Turfgrass Research Unit. a,b

Chemical			Plant-back interval	
Treatment	Rate	1 WAT <sup>c</sup>	2 WAT	3 WAT
	kg ai/ha		cm	
Oxadiazon	3.36	107 A	88 A	38 B
EPTC	7.84	87 A	64 A	24 B
EPTC + Dazomet	7.84 + 112	91 A	72 B	39 B
EPTC + Dazomet	7.84 + 224	98 A	80 B	32 B
EPTC + Dazomet	7.84 + 336	119 A	76 B	44 B
EPTC + Dazomet	7.84 + 448	110 A	73 B	39 C
Dazomet	112	94 A	64 B	41 B
Dazomet	224	88 A	59 B	34 B
Dazomet	336	83 A	74 A	43 A
Dazomet	448	86 A	69 A	37 B
<sup>a</sup> Zoysiagrass planti	ng dates were 1	4, 21, and 28 Augus	st, 2009. Three longes	t stolons were

measured on 20 January, 2010. Since fixed effect treatment and treatment × plant-back interval failed to be significant at 5% level, no treatment comparisons were performed within each plant-

back interval.

<sup>&</sup>lt;sup>b</sup> Means followed by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviation: WAT, Weeks After Treatment.

Table 8. BK-7 zoysiagrass dry weight as affected by three plant-back intervals within soil chemical treatment, 2009. AU Turfgrass Research Unit.<sup>a,b</sup>

Chamiaal	_		Plant-back interval	
Chemical Treatment	Rate	1 WAT <sup>c</sup>	2 WAT	3 WAT
	kg ai/ha		g dry wt/plot <sup>d</sup> ——	
Oxadiazon	3.36	42.1 A	34.5 A	15.1 B
EPTC	7.84	34.4 A	25.0 A	9.5 B
EPTC + Dazomet	7.84 + 112	35.8 A	28.4 B	15.5 B
EPTC + Dazomet	7.84 + 224	38.7 A	31.3 B	12.7 B
EPTC + Dazomet	7.84 + 336	47.0 A	29.9 B	17.4 B
EPTC + Dazomet	7.84 + 448	43.1 A	28.7 B	15.3 C
Dazomet	112	37.0 A	25.0 B	16.2 B
Dazomet	224	34.7 A	23.2 B	13.3 B
Dazomet	336	32.7 A	29.2 A	16.9 A
Dazomet	448	34.0 A	27.3 A	14.4 B

<sup>&</sup>lt;sup>a</sup> Zoysiagrass planting dates were 14, 21 and 28 August, 2009; harvested on 25 May, 2010; oven dried at 60 C for 4 days before measuring dry weight. Since fixed effect treatment and treatment × plant-back interval failed to be significant at 5% level, no treatment comparisons were performed within each plant-back interval.

<sup>&</sup>lt;sup>b</sup> Means followed by same upper-case letter within a row and soil chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviation: WAT, Weeks After Treatment.

<sup>&</sup>lt;sup>d</sup> Five rooted plants per plot.

Table 9. Straight Eight cucumber dry weight as affected by soil chemical treatments and plant-back intervals, 2009. AU Turfgrass Research Unit.<sup>a,b</sup>

Chemical				Plant-back interv	al		
Treatment	Rate	1 V	VAT <sup>c</sup>	2 WAT	3 v	3 WAT	
	kg ai/ha		g/seedling				
Oxadiazon	3.36	0 c	A	0.21 c A	0.17 a	A	
EPTC	7.84	0.86 b	A	0.63 bc A	0.36 a	A	
EPTC + Dazomet	7.84 + 112	0.99 b	A	0.81 abc A	0.35 a	A	
EPTC + Dazomet	7.84 + 224	1.02 b	A	0.86 abc A	0.41 a	Α	
EPTC + Dazomet	7.84 + 336	1.09 b	A	1.34 ab A	0.56 a	A	
EPTC + Dazomet	7.84 + 448	0.84 b	AB	1.43 ab A	0.48 a	В	
Dazomet	112	1.89 ab	A	0.75 abc AB	0.40 a	В	
Dazomet	224	2.42 a	A	0.91 abc B	0.39 a	В	
Dazomet	336	2.88 a	A	1.13 abc B	0.58 a	В	
Dazomet	448	2.53 a	A	1.80 a AB	0.57 a	В	

<sup>&</sup>lt;sup>a</sup> Cucumber seed planting dates were 14, 21, and 28 August, 2009; six seeds per plot were planted in 3 hills with 2 seeds per hill; harvested on 4, 11 and 18 September, 2009; oven dried at 60 C for 4 days before measuring dry weight.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviation: WAT, Weeks After Treatment.

Table 10. Average length of Tifway bermudagrass stolons as affected by soil chemical treatments and plant-back intervals, 2009. Gulf Coast Research and Extension Center. a,b

Chemical				Plant-back	interval		
Treatment <sup>c</sup>	Rate	1 W	ALT <sup>c</sup>	2 WA	LT	3 W	ALT
	kg ai/ha			cn	n		
EPTC	7.84	12 c	A	9 c	A	9 ab	A
EPTC fb 2,4-D	7.84 fb 2.24	11 c	A	10 c	A	8 b	A
Dazomet fb EPTC	224 fb 7.84	17 bc	A	16 abc	A	16 a	A
Dazomet fb EPTC	224 fb 7.84						
fb 2,4-D	fb 2.24	11 c	В	18 ab	A	16 a	AB
Dazomet fb 2,4-D	224 fb 2.24	22 ab	Α	21 a	A	12 ab	В
Oxadiazon	3.36	16 bc	AB	20 a	A	13 ab	В
2,4-D	2.24	24 a	A	12 bc	В	10 ab	В

<sup>&</sup>lt;sup>a</sup> Bermudagrass planting dates were 15, 22, and 29 July, 2009. Stolon length was measured on the 12, 19, and 26 August, 2009 which provided 1-month growth for each plant-back interval after the 2,4-D application. All stolons greater than 2.54 cm were measured.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

Table 11. Total stolon length (TSL) of Tifway bermudagrass as affected by soil chemical treatments and plant-back intervals, 2009. Gulf Coast Research and Extension Center. ab

		Plant-back interval					
Chemical		1 WA	LT <sup>c</sup>	2 WAI	LT	3 W.	ALT
Treatment <sup>c</sup>	Rate	T	SL	TS	SL	T	SL
	kg ai/ha	cm		cm		cm	
EPTC	7.84	391 b	A	282 c	A	168 bc	A
EPTC fb 2,4-D	7.84 fb 2.24	286 b	A	246 с	A	138 bc	A
Dazomet fb EPTC	224 fb 7.84	537 b	A	523 bc	A	481 a	A
Dazomet fb EPTC	224 fb 7.84						
fb 2,4-D	fb 2.24	337 b	В	697 ab	A	382 ab	В
Dazomet fb 2,4-D	224 fb 2.24	1144 a	A	919 a	A	281 abc	В
Oxadiazon	3.36	308 b	В	745 ab	A	426 a	В
2,4-D	2.24	856 a	A	349 c	В	121 c	В

<sup>&</sup>lt;sup>a</sup>Bermudagrass planting dates were 15, 22, and 29 July, 2009. Stolon length was measured on the 12, 19, and 26 August, 2009 which provided 1-month growth for each plant-back interval after the 2,4-D application. All stolons greater than 2.54 cm were measured.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

Table 12. Percent ground cover for Tifway bermudagrass as affected by soil chemical treatments and plant-back intervals, 2009. Gulf Coast Research and Extension Center. a,b

Chemical				Plant-back	interval		
Treatment <sup>c</sup>	Rate	1 W	ALT <sup>c</sup>	2 WA	2 WALT		ALT
	kg ai/ha		——————————————————————————————————————				
EPTC	7.84	44 ab	A	27 d	В	35 ab	AB
EPTC fb 2,4-D	7.84 fb 2.24	46 ab	A	28 d	В	33 ab	В
Dazomet fb EPTC	224 fb 7.84	55 ab	A	42 bcd	В	43 a	AB
Dazomet fb EPTC	224 fb 7.84						
fb 2,4-D	fb 2.24	55 ab	A	51 ab	A	44 a	A
Dazomet fb 2,4-D	224 fb 2.24	60 a	A	46 abc	В	33 ab	C
Oxadiazon	3.36	50 ab	AB	62 a	A	39 a	В
2,4-D	2.24	40 b	A	32 cd	AB	25 b	В

<sup>&</sup>lt;sup>a</sup>Bermudagrass planting dates were 15, 22, and 29 July, 2009. Pictures were taken on 9 October, 2009. A camera was mounted on a portable light box that provided the same quality and

intensity of illumination for each photograph.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

<sup>&</sup>lt;sup>d</sup> % ground cover is the ratio of green pixels selected by previously set color thresholds, to total pixel count of the image.

Table 13. Average length of BK-7 zoysiagrass stolons as affected by soil chemical treatments and plant-back intervals, 2009. Gulf Coast Research and Extension Center. <sup>a,b</sup>

Chemical	Plant-back interval						
Treatment <sup>c</sup>	Rate	1 WALT <sup>c</sup>		2 WALT		3 WA	LT
	kg ai/ha			cm			
EPTC	7.84	4 a	A	4 a	A	5 a	A
EPTC fb 2,4-D	7.84 fb 2.24	4 a	A	4 a	A	5 a	A
Dazomet fb EPTC	224 fb 7.84	5 a	A	5 a	A	4 a	A
Dazomet fb EPTC	224 fb 7.84						
fb 2,4-D	fb 2.24	5 a	A	5 a	A	5 a	A
Dazomet fb 2,4-D	224 fb 2.24	4 a	A	4 a	A	5 a	A
Oxadiazon	3.36	5 a	A	4 a	A	5 a	A
2,4-D	2.24	4 a	A	3 a	A	4 a	A

<sup>&</sup>lt;sup>a</sup>Zoysiagrass planting dates were 15, 22, and 29 July, 2009. Stolon length was measured on the

<sup>12, 19,</sup> and 26 August, 2009 which provided 1-month growth for each plant-back interval after the 2,4-D application. All stolons greater than 2.54 cm were measured. All three fixed effects (treatment, plant-back interval and treatment  $\times$  plant-back interval) failed significant test at 5% level.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by the same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

Table 14. Total stolon length (TSL) of BK-7 zoysiagrass as affected by soil chemical treatments and plant-back intervals, 2009. Gulf Coast Research and Extension Center. ab

		Plant-back interval							
Chemical		1 WA	LT <sup>c</sup>	2 WALT	3 WALT				
Treatment <sup>c</sup>	Rate	TS	SL	TSL	TSL				
	kg ai/ha	cm		cm	cm				
EPTC	7.84	37 b	В	79 abc B	140 a A				
EPTC fb 2,4-D	7.84 fb 2.24	50 b	В	51 bc B	105 a A				
Dazomet fb EPTC	224 fb 7.84	163 a	A	107 ab A	114 a A				
Dazomet fb EPTC	224 fb 7.84								
fb 2,4-D	fb 2.24	133 a	A	113 a A	128 a A				
Dazomet fb 2,4-D	224 fb 2.24	67 b	A	78 abc A	126 a A				
Oxadiazon	3.36	53 b	A	25 c A	47 b A				
2,4-D	2.24	48 b	A	42 c A	32 b A				

<sup>&</sup>lt;sup>a</sup> Zoysiagrass planting dates were 15, 22, and 29 July, 2009. Stolon length was measured on the 12, 19, and 26 August, 2009 which provided 1-month growth for each plant-back interval after

the 2,4-D application. All stolons greater than 2.54 cm were measured.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

Table 15. Percent ground cover for BK-7 zoysiagrass as affected by soil chemical treatments and plant-back intervals, 2009. Gulf Coast Research and Extension Center.<sup>a,b</sup>

Chemical	Plant-back interval						
Treatment <sup>c</sup>	Rate	1 WALT <sup>c</sup>		2 WALT		3 WA	LT
	kg ai/ha	——————————————————————————————————————					
EPTC	7.84	18 a	A	15 a	A	17 a	A
EPTC fb 2,4-D	7.84 fb 2.24	19 a	A	16 a	A	15 a	A
Dazomet fb EPTC	224 fb 7.84	22 a	A	14 a	A	15 a	A
Dazomet fb EPTC	224 fb 7.84						
fb 2,4-D	fb 2.24	15 a	A	17 a	A	15 a	A
Dazomet fb 2,4-D	224 fb 2.24	19 a	A	14 a	A	16 a	A
Oxadiazon	3.36	16 a	A	16 a	A	14 a	A
2,4-D	2.24	18 a	A	16 a	A	14 a	A

<sup>&</sup>lt;sup>a</sup> Zoysiagrass planting dates were 15, 22, and 29 July, 2009. Pictures were taken on 9 October,

2009. A camera was mounted on a portable light box that provided the same quality and intensity of illumination for each photograph. All three fixed effects (treatment, plant-back interval and treatment × plant-back interval) failed significant test at 5% level.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

<sup>&</sup>lt;sup>d</sup> % ground cover is the ratio of green pixels selected by previously set color thresholds, to total pixel count of the image.

Table 16. Average length of Palmetto St. Augustinegrass stolons as affected by soil chemical treatments and plant-back intervals, 2009. Gulf Coast Research and Extension Center. a,b

Chemical		Plant-back interval						
Treatment <sup>c</sup>	Rate	1 W	ALT	2 W A	ALT	3 V	VALT	
	kg ai/ha			C1	m			
EPTC	7.84	12 a	A	14 a	A	9 a	A	
EPTC fb 2,4-D	7.84 fb 2.24	11 a	A	11 a	A	9 a	A	
Dazomet fb EPTC	224 fb 7.84	18 a	A	16 a	A	12 a	A	
Dazomet fb EPTC	224 fb 7.84							
fb 2,4-D	fb 2.24	14 a	A	13 a	A	10 a	A	
Dazomet fb 2,4-D	224 fb 2.24	15 a	A	13 a	A	12 a	A	
Oxadiazon	3.36	12 a	A	11 a	A	15 a	A	
2,4-D	2.24	10 a	A	13 a	A	12 a	A	

<sup>&</sup>lt;sup>a</sup> St.Augustinegrass planting dates were 15, 22, and 29 July, 2009. Stolon length was measured on the 12, 19, and 26 August, 2009 which provided 1-month growth for each plant-back interval after the 2,4-D application. All stolons greater than 2.54 cm were measured. All three fixed effects (treatment, plant-back interval and treatment × plant-back interval) failed significant test at 5% level.

<sup>&</sup>lt;sup>b</sup> Means followed by the same lower-case letter within a column and plant-back interval or by the same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

Table 17. Total stolon length (TSL) of Palmetto St. Augustinegrass as affected by three plant-back intervals within soil chemical treatment, 2009. Gulf Coast Research and Extension Center. ab

	_	Plant-back interval							
Chemical	_	1 WA	LT <sup>c</sup>	2 WA	LT	3 WALT			
Treatment <sup>c</sup>	Rate	TSL		TSL		TSL			
	kg ai/ha	cm		cm		cm			
EPTC	7.84	151	A	85	A	72 B			
EPTC fb 2,4-D	7.84 fb 2.24	161	A	143	A	93 A			
Dazomet fb EPTC	224 fb 7.84	236	AB	356	A	74 B			
Dazomet fb EPTC	224 fb 7.84								
fb 2,4-D	fb 2.24	216	A	247	A	76 A			
Dazomet fb 2,4-D	224 fb 2.24	176	В	340	A	124 B			
Oxadiazon	3.36	125	A	128	A	135 A			
2,4-D	2.24	93	A	93	A	180 A			

<sup>&</sup>lt;sup>a</sup> St. Augustinegrass planting dates were 15, 22, and 29 July, 2009. Stolon length was measured on the 12, 19, and 26 August, 2009 which provided 1-month growth for each plant-back interval after the 2,4-D application. All stolons greater than 2.54 cm were measured. Since fixed effect treatment and treatment × plant-back interval failed to be significant at 5% level, no treatment comparisons were performed within each plant-back interval.

<sup>&</sup>lt;sup>b</sup> Means followed by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

Table 18. Percent ground cover for Palmetto St. Augustinegrass as affected by three plant-back intervals within soil chemical treatment, 2009. Gulf Coast Research and Extension Center.<sup>a,b</sup>

Chamical		Plant-back interval						
Chemical Treatment <sup>c</sup>	Rate	1 WALT <sup>c</sup>	2	2 WALT		3 WALT		
	kg ai/ha							
EPTC	7.84	23	Α	18	AB	11	В	
EPTC fb 2,4-D	7.84 fb 2.24	23	A	18	A	14	A	
Dazomet fb EPTC	224 fb 7.84	28	A	27	A	15	В	
Dazomet fb EPTC	224 fb 7.84							
fb 2,4-D	fb 2.24	25	A	24	A	16	A	
Dazomet fb 2,4-D	224 fb 2.24	22	AB	24	A	14	В	
Oxadiazon	3.36	25	A	18	A	17	A	
2,4-D	2.24	17	A	16	A	21	A	

<sup>&</sup>lt;sup>a</sup> St. Augustinegrass planting dates were 15, 22, and 29 July, 2009. Pictures were taken on 09

October, 2009. A camera was mounted on a portable light box that provided the same quality and intensity of illumination for each photograph. Since fixed effect treatment and treatment × plant-back interval failed to be significant at 5% level, no treatment comparisons were performed within each plant-back interval.

<sup>&</sup>lt;sup>b</sup> Means followed by same upper-case letter within a row and chemical treatment are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> Abbreviations: fb, followed by; WALT, Weeks After Last Treatment which corresponded to the 2,4-D application.

<sup>&</sup>lt;sup>e</sup> % ground cover is the ratio of green pixels selected by previously set color thresholds, to total pixel count of the image.

Table 19. EPTC degradation under field condition as affected by sampling date and treatment history after a 7.84 kg ai/ha application, 2010. AU Turfgrass Research Unit. ab

Chamiaal		Tuestusent	Sampling date					
Chemical	Data	Treatment	1 DAT <sup>d</sup>	3 DAT	7 DAT			
Treatment	Rate	history <sup>c</sup>	1 DA1	3 DA I	7 DAT			
	kg ai/ha			ppm				
EPTC	7.84	2 years	6.152 a	4.027 a	0.013 a			
EPTC + Dazomet	7.84 + 448	2 years	2.866 bc	1.554 c	0.012 a			
EPTC	7.84	1 year	3.993 b	2.444 b	0.009 a			
EPTC + Dazomet	7.84 + 448	1 year	2.593 c	1.449 c	0.020 a			

<sup>&</sup>lt;sup>a</sup> EPTC was applied to soil on 23 July, 2010 followed by power tiller incorporation and six mm irrigation immediately. Soil was sampled from the surface to a depth of 7.6 cm by hand using a small handhold-shovel. Soil samples were extracted in ethyl acetate by a wrist-action shaker and analyzed by GC-MS.

<sup>&</sup>lt;sup>b</sup> Means followed by same lower-case letter within a column and sampling date are not significant at 5% level according to Fisher's LSD test.

<sup>&</sup>lt;sup>c</sup> 2 years treatments history plots received either EPTC or EPTC + Dazomet in both 2008 and 2009; 1 year treatment history plots received either EPTC or EPTC + Dazomet only in 2009.

<sup>&</sup>lt;sup>d</sup> Abbreviation: DAT, Days After Treatment.