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Effect of phenyl, allyl, and methyl isothiocyanate on *Cyperus rotundus* tubers under LDPE and VIF mulch

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A B S T R A C T

Cyperus rotundus (Purple nutsedge) is the most troublesome weed of vegetable crops in the US and a major limitation to the adoption of methyl bromide alternatives due to their ineffectiveness on this weed. Thus, other effective alternatives are needed. Greenhouse studies were conducted to determine the effect of phenyl, allyl, and methyl isothiocyanate (ITC) at two concentrations (1000 and 3000 nmol g^{-1} in dry soil) on *C. rotundus* tubers under low-density polyethylene (LDPE) and virtually impermeable film (VIF) mulch. ITC treated soil containing *C. rotundus* tubers was filled in glass jars and covered with LDPE or VIF mulch for 21 days, followed by nutsedge tuber viability evaluations. Efficacy of all three ITCs increased with increase in concentration from 1000 to 3000 nmol g^{-1}. All ITCs significantly reduced tuber viability, tuber dry weight, and shoot emergence, but methyl ITC was most effective followed by allyl ITC followed by phenyl ITC. Mulch type did not affect efficacy of allyl and methyl ITC, but phenyl ITC efficacy against *C. rotundus* was improved by using VIF mulch over LDPE mulch. Overall, methyl ITC at 3000 nmol g^{-1} was the most efficacious control option among three ITC tested regardless of mulch type.

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1. Introduction

Cyperus rotundus is one of the most problematic weeds in polyethylene-mulched vegetable crops where few effective means of control other than methyl bromide are available. Depending upon infestation level, *C. rotundus* can cause yield losses from 43 to 100% in different vegetable crops (Morales-Payan et al., 1996, 1997a, 1997b, 1998; Santos et al., 1998; William and Warren, 1975). A single tuber of *C. rotundus* can produce 530 tubers in 13 weeks (Webster and Grey, 2014). Likewise, a single tuber of *C. rotundus* can produce up to 300 shoots m^{-2} during a single growing season (Webster, 2000), and a *C. rotundus* patch originating from a single tuber can expand up to 8 m^2 (Webster, 2005). The number of nutsedge shoots in most fields is so high that hand removal is not feasible. Furthermore, hand removal of shoots is a temporary solution due to the rapid regrowth of shoots from underground tubers which would require frequent removal to prevent weed interference with crops. Once established, *C. rotundus* is difficult to eradicate because of dormant tubers in soil (Bangarwa et al., 2008; Reddy and Bendixen, 1989; Santos et al., 1997). In addition to competitive ability, *C. rotundus* tubers and foliage possess allelopathic properties (Friedman and Horowitz, 1971). Unlike all other weeds, nutsedge seedlings readily penetrate plastic mulch destroying the mulch in addition to reducing crop yield and harvest efficiency (Chase et al., 1998). Due to above said reasons, methyl bromide has been routinely relied upon by vegetable producers to minimize *C. rotundus* interference, achieve maximum yields, and allow efficient harvest of a high quality crop, in addition to control of other weeds and pests in plasticulture production. This illustrates the need for highly efficacious alternatives for nutsedge control with the loss of methyl bromide.

Volatile biocidal compounds like isothiocyanates (ITCs) beneath plastic have broad-spectrum activity on weeds, insects, diseases, and nematodes. ITCs belong to a family of chemicals composed of $-N{=}C{=}S$ bonded to a variable side chain (R) (Fenwick et al., 1983). In nature, ITCs are released by degradation of glucosinolates (GSLs), which are produced by Brassicaceae and some other plant families. However, several ITCs are also synthetically produced these days. ITCs possess biocidal properties and have shown activity against several soil-borne pests (Brown and Morra, 1995; Matthiessen and Shackleton, 2005; Mattner et al., 2008; Zasada and Ferris, 2004). The herbicidal properties of ITCs have been confirmed against certain plant and weed species (Al-Khatib et al., 1997; Bangarwa et al., 2010; Bangarwa and Norsworthy, 2014; Bell and Muller,

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1973; Bialy et al., 1990; Norsworthy and Meehan, 2005a, b; Petersen et al., 2001; Teasdale and Taylorson, 1986; Vaughn and Boydston, 1997). The actual mechanism of ITC inhibition of seed germination is unknown but it is suspected to be an interaction with seed enzymes (Drobinca et al., 1977). Low concentration of ITCs can induce secondary dormancy, while higher concentrations can cause irreversible reaction with enzymes and make seed non-viable (Petersen et al., 2001). Although, the basic structure of ITCs is similar, a slight variation in side chain can change ITC molecular structure and in turn physical, chemical, and biological properties. Petersen et al. (2001) showed that aromatic ITCs are generally more effective than aliphatic ITCs but converse was true in another study (Borek et al., 1995). However, mixed results were reported by Norsworthy and Meehan (2005a,b) and Norsworthy et al. (2006). Several synthetic ITCs have been available, but the biological activity of each ITC has not been completely evaluated against *C. rotundus*. In general, ITCs are unstable in soil and >90% of ITCs were volatilized within 24 h (Brown and Morra, 1995). Therefore, ITC retention and in turn efficacy could be improved with the use of low permeability plastic mulches in field conditions. It would be beneficial to test efficacy of ITCs under low and high permeability plastic mulches, so that appropriate application methodology could be designed based on ITC type.

Greenhouse experiments were conducted to evaluate herbicidal activity of three ITCs: allyl ITC (C_4H_5NS; molecular wt. = 99.16 g/mol), phenyl ITC (C_7H_5NS; molecular wt. = 135.19 g/mol), and methyl ITC (C_2H_3NS; molecular wt. = 73.12 g/mol). These synthetic ITCs investigated in this research are currently only available in analytical quantities which will likely result in their cost being substantial if applied under field conditions. However, increased production of these compounds would likely result in costs more appropriate for the vegetable systems in which that may be used. One such ITC that is already in commercial use is methyl ITC, which is generated from a commercially available fumigant (metam sodium). Furthermore, the ITCs evaluated in this research have much lower human toxicity compared with methyl bromide. The overall goal of this research was to evaluate the herbicidal activity of phenyl, allyl, and methyl ITC at two concentrations under LDPE and VIF mulch on *C. rotundus* tubers.

2. Materials and methods

Greenhouse studies were conducted in Fall 2008 and Spring 2009 at Fayetteville, AR (36° 5′, 55.7″ N, 94° 1′, 44.5″ W), to evaluate phenyl, allyl, or methyl ITC efficacy against *C. rotundus* tubers. The soil type used for these studies was a Pembroke silt loam (fine-silty, mixed, active, nonacid, mesic Mollic Paleudalfs) with 2.0% organic matter and a pH of 6.0. Soil for these studies was collected in Fall 2008 from Arkansas Agricultural Research and Extension Center at Fayetteville, AR and stored at room temperature before use. For this, 1000 g of oven-dried soil was treated with each ITC to produce a concentration of 1000 and 3000 nmol g^{-1} in dry soil. These rates were determined by considering labeled rates of the commercial fumigant and methyl ITC generator metam sodium. When applied at a label application rate of 360 kg ha^{-1} with an incorporation depth of 7.5 cm, metam sodium can produce up to 2500 nmol g^{-1} of methyl ITC (assuming 100% conversion and soil bulk density of 1.48 g cm^{-3}). Each ITC was mixed with 100 mL of distilled water for uniform distribution of ITC in soil. Because ITCs are less polar than water, each ITC was vigorously mixed in water by continuous agitation to create a uniform suspension of ITC in water. This suspension was then intermittently poured in to plastic bags containing soil and thoroughly mixed for uniform treatment of soil. Twenty *C. rotundus* tubers were mixed in the treated soil, and the soil was immediately transferred to 946-mL glass jars, and the jars were covered with a LDPE (black, embossed, 1.0 mil thick) or VIF (black/white, smooth, 1.3 mil thick) mulch. The jars were then placed in a greenhouse for a period of 21 days. This time period was selected because growers generally use 21 days period for methyl bromide fumigation. In addition, a non-treated control treatment was also established in which tubers were not treated with ITC. Greenhouse conditions were 32/22 °C day/night temperatures and 14/10 h day/night light period. After 21 d of exposure period, tubers were removed from the jars, washed, and tested for germination in pots filled with sterilized soil for 4 weeks in the greenhouse under the same conditions described above. After 4 weeks, total shoot numbers emerged from sprouted tubers were counted. All sprouted tubers were then counted and removed from the pots. All non-sprouted tubers were removed from the pots and tested for viability based on firmness and pulp color. Tubers that were rotten and tubers that were firm but with <10% white pulp inside were considered non-viable. Viable tubers were oven-dried to obtain total viable tubers dry weight. Experimental design was a randomized complete block design with four replications. Treatments were arranged in a 3 factor factorial structure of ITC type, concentration, and mulch type. Data from untreated control were excluded from analysis. The control treatment was considered as a reference treatment and tuber viability, tuber dry weight, and shoot emergence in this treatment were considered as 100%. Data were subjected to ANOVA, with runs and replications treated as random effects whereas ITC type, concentration, and mulch were fixed effects. Tuber viability, tuber dry weight, and shoot emergence (% of untreated control) means were compared using Fischer's protected LSD at 5% level of significance.

3. Results and discussion

ANOVA indicated that tuber viability, tuber dry weight, and shoot emergence was influenced by the interaction of ITC and mulch type and main effects of ITC type, concentration, and mulch type. Averaged over concentrations, tuber viability was lower under VIF mulch (24.6%) compared to LDPE mulch (32.2%) for phenyl ITC (Table 1). Similar trends were observed with tuber dry weight (30.9 vs 36.7%) and shoot emergence (22.6 vs 28.4%) data. This effect could be due to higher retention (reduced volatilization) of phenyl ITC under VIF mulch compared to LDPE mulch. This higher retention would have maintained higher concentration and longer exposure period of phenyl ITC with tubers and possibly have enhanced phenyl ITC efficacy under VIF mulch. This higher retention of phenyl ITC under VIF mulch may be attributed to the polyamide barrier embedded in the polyethylene sheets of VIF mulch, which makes it less permeable than LDPE mulch (Yates et al., 2002). However, mulch type did not affect tuber viability, tuber dry weight, and shoot emergence for allyl and methyl ITC (Table 1). In previous research, Bangarwa et al. (2010) reported that half-life of phenyl ITC was increased by 2.8 days by covering the treated soil with VIF mulch instead of LDPE mulch. However, the half-life of allyl ITC was increased only by 0.4 days under VIF mulch over LDPE mulch (Bangarwa and Norsworthy, 2014). Thus, this differential retention of phenyl ITC could have caused differential *C. rotundus* tuber and shoot suppression under two mulch types. The above results are in agreement with Teasdale and Taylorson (1986) who suggested that ITC concentration and length of exposure are critical for weed suppression. Therefore, these results suggest that phenyl ITC efficacy against *C. rotundus* may be enhanced by covering the treated soil with low permeability plastic mulches under field conditions.

In general, tuber viability, tuber dry weight, and shoot emergence were lowest from *C. rotundus* tubers treated with methyl ITC followed by allyl ITC followed by phenyl ITC (Table 1). This shows

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