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10-16-2023

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RESEARCH ARTICLE



# Assessing the toxicity of selected pesticides to *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) pupae as a first step in the development of a potential novel deployment programme

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## ABSTRACT

*Trichogramma* spp. have been used or have been proposed for use in biological control programmes of lepidopteran pests, including the European corn borer, *Ostrinia nubilalis*, the cotton bollworm, *Helicoverpa armigera*, and the spruce budworm, *Choristoneura fumiferana*. Releases are typically made by placing cards with parasitised eggs at set points in the field. However, this method can be cost-prohibitive due to its laborious nature. As a result, labour-saving mechanised release programmes have been developed, including distribution by spray equipment. However, few have investigated applying *Trichogramma* with a standard pesticide application (i.e. ‘tank mix’). As a first step to ascertain the feasibility of such a system, we observed the effect of immersion of *T. ostriniae* pupae in field-relevant concentrations of eight herbicides (bentazon, clethodim, fomesafen, quizalofop, Glacial acetic acid, glyphosate, imazamox, imazethapyr), three fungicides (Copper Hydroxide, flutriafol, and penthiopyrad), one insecticide (K<sup>+</sup> salt of fatty acid), and five adjuvants (Ammonium Sulfate, Crop Oil Concentrate, Methylated seed oil, Non-ionic surfactant, and Urea-Ammonium Nitrate) on the emergence of *T. ostriniae* adults. The herbicides GAA and clethodim; the fungicides copper hydroxide, flutriafol, and penthiopyrad; and the adjuvants COC, MSO, and NIS all reduced *T. ostriniae* emergence compared to a water control. No emergence was observed with exposure to quizalofop, GAA, or K<sup>+</sup> salt of fatty acid treatments. Other treatments did not affect emergence compared to water. Thus, deploying *Trichogramma* with a standard pesticide application may be a feasible labour-saving distribution method that warrants further investigation.

## ARTICLE HISTORY

Received 18 July 2023  
Returned 19 September 2023  
Accepted 16 October 2023

## KEYWORDS

*Trichogramma* release; agrochemicals; labour-saving; broadcast application; biocontrol; integrated pest management

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## Introduction

*Trichogramma* is a genus of gregarious endoparasitoid wasp widely used to control lepidopterous pests (Pinto & Stouthamer, 1994; Querino et al., 2010; Smith, 1994, 1996; Zang et al., 2021). At the turn of the millennium, *Trichogramma* spp. were released on over 32 million hectares of forest and cropland each year (VanLenteren, 2000). Although, this figure may be an overestimate as some of the hectareage were double or tipples counted in the former USSR (VanLenteren, 2000).

*Trichogramma* spp. have several attributes that make them ideal candidates for biological control. Benefits include ease of rearing and the destruction of the host prior to its damaging stage (Davies et al., 2011; Hassan, 1994; Hegazi et al., 2012; Oliveira et al., 2014; Pak, 1988; Sarwar & Salman, 2015; VanLenteren, 2000; Wang et al., 2014). Ease of rearing is facilitated by the use of factitious hosts such as *Corcyra cephalonica*, *Ephestia kuehniella*, and *Sitotroga cerealella* (Bigler et al., 1987; Dysart, 1973; El-Wakeil, 2007; Gowda et al., 2021; Hassan, 1993; Wang et al., 2014; Zang et al., 2021). Inundative releases of *Trichogramma* spp. have been made in various environments, including forestland, tree fruit, and field and vegetable crops (Hassan, 1993; Martel et al., 2021; Wang et al., 2014; Zang et al., 2021). *Trichogramma minutum* has been trialled for control of the spruce budworm, *Choristoneura fumiferana*, in North American forests (Martel et al., 2021; Smith et al., 1990). *Trichogramma maidis* has been used to control the European corn borer, *Ostrinia nubilalis*, in corn in Switzerland (Bigler, 1986). *Trichogramma chilonis* has been used to control *Helicoverpa armigera* (Masood et al., 2011). *Trichogramma ostriniae* has been trialled for control of the western bean cutworm *Striacosta albicosta* (Seaman, 2017). A more comprehensive list of *Trichogramma* uses is reported by Smith (1994) and Cluever (2023).

Usually, *Trichogramma* are released during their pupal stage inside the host egg. Host eggs are glued to a substrate such as cardboard, sometimes called a 'Trichocard,' which is then fastened to the plant (Dionisio & Calvo, 2022; Gagnon et al., 2017; Knutson, 1998; Sarwar & Salman, 2015; Schmidt et al., 2003; Smith, 1994; Zhan et al., 2021). Smith (1996) stated that at least six release points should be made per hectare. However, the minimum number of release points and the number released at each point varies (See Basso et al., 2020; Bueno et al., 2011; Chapman et al., 2009; Geremias & Parra, 2014; Hommay et al., 2002; Martel et al., 2021 for examples). Releases are often more effective if release points are spaced in such a way as to give a minimal distance between each point (Askar et al., 2018; Basso et al., 2020; Bueno et al., 2011; Tohamy & Kassem, 2007; Zachrisson & Parra, 1998). This spacing allows uniform population dispersion to be attained quickly (Geetha & Balakrishnan, 2010; Geremias & Parra, 2014; Gusev & Lebedev, 1988; Mahrugan et al., 2015; Smith, 1994). While this approach is technically simple to implement, it can be costly in terms of labour and may be difficult to achieve (Ables et al., 1979; Basso et al., 2020; Ji et al., 2022; Kienzle et al., 2012; Martel et al., 2021; Parra, 2010; Smith, 1996; Zang et al., 2021; Zhan et al., 2021).

Bigler (1986) stated that deploying *Trichogramma* on cards would require thirty minutes of labour per hectare in corn. So, this would cost the grower USD 3.63 ha<sup>-1</sup> if one assumes the U.S. 2022 federal minimum wage or CAD 7.78 ha<sup>-1</sup> if one assumes the Canadian 2022 federal minimum wage (Anonymous, 2021, 2022). However, others have estimated that it would take more time. Anonymous (1974) stated that it would

take one person an entire day to apply *Trichogramma* across ten hectares (as cited by Jones et al., 1977). BIRTHAL et al. (2000) stated that two *Trichogramma* releases would take 4.23 person-days ha<sup>-1</sup> in cotton. Jones et al. (1977) states that the lack of mechanised release methods is the main economic factor limiting the use of *Trichogramma*. Additionally, Stinner (1977) and Van Lenteren and Bueno (2003) posit that the primary limitation to inundative biological control is economic.

Releasing *Trichogramma* in capsules from crewed or uncrewed aircraft may save on labour during releases; however, this may still prove uneconomical due to the time and expense required to prepare the capsules (Ables et al., 1979; Zhan et al., 2021). Additionally, broadcast releases may offer greater uniformity and efficacy (Gardner & Giles, 1997; Mikhal'tsov & Purshkarev, 1981; Pas'ko et al., 1982). Thus, broadcast releases of loose *Trichogramma* may be advantageous (Smith, 1994).

Broadcast releases of biological control agents are often made with carriers to allow for a more precise dispersal. Carriers include sawdust, tobacco seed, water, bran, vermiculite, and hydrocolloid mixtures (Bouse et al., 1980; Gardner & Giles, 1997; Kienzle et al., 2012; Martel et al., 2021; Pas'ko et al., 1982; Reeves, 1975). Gardner and Giles (1997) proposed applying *T. pretiosum* through standard insecticide application equipment. Filippov (1989) stated that 140–150 hectares could be treated in 8 h through these methods. According to Dionne et al. (2018) application of *T. ostrinae* via boom sprayer may be 1.7 times faster than manual placement in sweet corn. Unfortunately, they note that spraying *T. ostrinae* costs 29.7% more than manual placement due to the need for additional *T. ostrinae* and adjuvants (Guar and Xanthan gum). Therefore, we propose using pre-existing herbicide or fungicide applications to distribute *T. ostrinae*.

Previous studies have assessed the toxicity of pesticides to *Trichogramma* (Bastos et al., 2006; Khan & Ruberson, 2017; Nusillard et al., 2023) and other egg parasitoids (Carmo et al., 2010; Turchen et al., 2016). For instance, Nusillard et al. (2023) assessed the trophic effects of copper sulfate on *T. cordubensis* reared on *Lobesia botrana*. Bueno et al. (2008) assessed the effect of 14 pesticides on the egg, larval, and pupal stages of *T. pretiosum* by immersion in solution for five seconds. Hassan et al. (1998) assessed the effect of 21 pesticides on *T. cacoeciae* by residue exposure and to pupae by spaying the host. They also assessed the effect of duration of toxicity on leaves. Bastos et al. (2006) assessed the effect of 20 pesticide formulations on *T. pretiosum* survival by immersing pupae in solution for five seconds. Khan and Ruberson (2017) assessed the effect of eleven pesticides on *T. pretiosum* by immersion of eggs, larvae, and pupae in solution for ten seconds. Turchen et al. (2016) assessed the effects of three insecticides on *Telonomus podisi*. Carmo et al. (2010) observed the effect of exposure to 28 pesticide residues on *T. remus* parasitism and emergence. Sub-lethal effects on egg parasitoids include a reduction in fecundity (Fontes et al., 2018; González et al., 2013; Turchen et al., 2016; Wang et al., 2012), longevity (Ribeiro et al., 2021; Wang et al., 2012), response to host volatiles (Bayram et al., 2010), and wing deformities (Tai et al., 2022; Xie et al., 2022). For a more detailed review of pesticide toxicity to *Trichogramma* see Cluever (2023).

However, these previous studies aimed not to evaluate the potential of a parasitoid and pesticide tank mix, but to assess the effect of pesticide exposure to *Trichogramma* already in the field. Thus, the authors did not expose *Trichogramma* to solutions for sufficient time to be relevant in a field-applied mixture.

The timing of standard pesticide applications may often align with the time when *Trichogramma* spp. can be used. For example, in western Nebraska, the pre-closure herbicide application in dry bean occurs in early July (NCL pers. observation). This application often aligns with the start of the western bean cutworm, *S. albicosta* flight (Cluever et al., 2021; Hanson et al. 2015). Additionally, corn fungicide applications may align with this timing (Jackson-Ziems, 2020).

*Trichogramma ostrinae* Pang et Chen is native to central and northern China (Li, 1994; Pang & Chen, 1974; Zong et al., 1988). This species has been used for inundative biological control of the Asian corn borer, *Ostrinia furnacalis*, and was imported to the United States in the 1990s (Lobdell et al., 2005; Wang et al., 2014). Since then, it has been used to control the European corn borer, *O. nubilalis*, in bell pepper, potato, sweet corn, and field corn in eastern North America (Chapman et al., 2009; Gagnon et al., 2017; Gardner et al., 2012; Kuhar et al., 2004; Wright et al., 2001).

Releasing *T. ostrinae* through a planned pesticide application could be an effective, low-cost, time-saving novel application process. As a first step in testing this novel distribution method, we assessed how immersion of *T. ostrinae* pupae in pesticide solutions affects emergence rates. We posit that most of the herbicides and fungicides will not affect *T. ostrinae* emergence as they are generally less harmful than insecticides and are protected by their host's egg (Rakes et al., 2021; Smith, 1996). However oil-based pesticides may have less trouble penetrating the waxy host egg (Cónsoli et al., 1999) as noted by Khan and Ruberson (2017) for S-metolachlor.

## Materials and methods

### Source of *Trichogramma ostrinae*

*Trichogramma ostrinae* pupae were obtained from a colony maintained at the Panhandle Research, Extension, and Education Center (PREEC) in Scottsbluff, Nebraska. The colony was maintained on sterilised *Ephestia kuehniella* Zeller eggs (Beneficial Insectary, Redding, CA) in a growth chamber with an 18:6 (L:D) h photoperiod at  $\approx 25^{\circ}\text{C}$ . The *T. ostrinae* used to intimate the PREEC colony were procured from the Cornell University Insectary in 2017. The Cornell University Insectary colony was started from *T. ostrinae* procured from the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) Mission Biological control center in 1993. The USDA obtained *T. ostrinae* from northern China (VanLenteren, 2000). Many other studies have used this isolate (Chapman et al., 2009; Gardner et al., 2012; Kuhar et al., 2002, 2004; Wright et al., 2001). All *T. ostrinae* used in this experiment were pupae inside loose *E. kuehniella* eggs.

### Preparation of treatments

Commercial formulations of herbicides, fungicides, and one insecticide (Table 1) were mixed with filtered (reverse osmosis) water at field-relevant concentrations. Pesticides and adjuvants were chosen based upon the level of their usage in the United States especially in dry edible bean and corn. The concentrations were chosen based upon the minimum and maximum that one could reasonably expect to see in the field

**Table 1.** Treatments (products and concentrations).

Type	Treatment	Trade name	Chemical class <sup>a</sup>	Concentration <sup>b</sup>	Field rate (g ai ha <sup>-1</sup> ) <sup>c</sup>
Control	Water		–	–	–
	Air		–	–	–
Adjuvant	Ammonium sulfate	AMS	–	17.97 g l <sup>-1</sup>	1681.2
	Crop oil concentrate	COC	–	0.5% v v <sup>-1</sup>	–
			–	1.0% v v <sup>-1</sup>	–
	Methylated seed oil	MSO	–	0.5% v v <sup>-1</sup>	–
			–	1.0% v v <sup>-1</sup>	–
	Non-ionic surfactant	NIS	–	0.5% v v <sup>-1</sup>	–
Herbicide	Urea-ammonium nitrate	UAN	–	2.5% v v <sup>-1</sup>	–
			–	5.0% v v <sup>-1</sup>	–
	Quizalofop	Assure II	1	0.21 g l <sup>-1</sup>	19.3
	Bentazon	Basagran	6	3.59 g l <sup>-1</sup>	336.3
				11.98 g l <sup>-1</sup>	1120.9
	Glacial acetic acid	GAA	NA	104.57 g l <sup>-1</sup>	9781.6
				209.17 g l <sup>-1</sup>	19565.7
	Imazethapyr	Pursuit	2	0.28 g l <sup>-1</sup>	26.3
				0.56 g l <sup>-1</sup>	52.5
	Imazamox	Raptor	2	0.19 g l <sup>-1</sup>	17.5
Fungicide	Fomesafen	Reflex	14	0.37 g l <sup>-1</sup>	35.0
				1.5 g l <sup>-1</sup>	140.1
				4.5 g l <sup>-1</sup>	420.9
	Glyphosate	Round up	9	5.66 g l <sup>-1</sup>	529.8
		Powermax		16.50 g l <sup>-1</sup>	1543.4
	Clethodim	Select Maxx	1	0.41 g l <sup>-1</sup>	38.2
	Copper hydroxide	Kocide 3000	M1	7.2 g l <sup>-1</sup>	673.6
				50.41 g l <sup>-1</sup>	4715.1
Insecticide	Flutriafol	Topguard	3	0.34 g l <sup>-1</sup>	31.9
				1.36 g l <sup>-1</sup>	127.5
	Penthiopyrad	Vertisan	7	0.78 g l <sup>-1</sup>	73.1
	K Salt of fatty acid	Safer		1.99% v v <sup>-1</sup>	–

<sup>a</sup>Mode of action #s.

<sup>b</sup>Concentrations are based upon maximum and minimum one would reasonable see in the field.

<sup>c</sup>Assuming a carrier volume of 15.3 l ha<sup>-1</sup> (10 gal ac<sup>-1</sup>)<sup>60,61</sup>.

(Knezevic, 2018; NCL personal Observation). The mixtures were placed in 20 ml glass scintillation vials. Then 0.1 g of *T. ostriniae* pupae inside an *E. kuehniella* host were added to each vial. Vials were placed in a fume hood arranged in an RCBD design for the duration of exposure. Each treatment was replicated four times with two separate *T. ostriniae* cohorts for a total of eight replications.

An aliquot (~1–2 ml) of *T. ostriniae* pupae was pipetted from vials at 1.5, 3, 6.5, 9, 12, and 22.5 h of treatment exposure and placed onto a bleached white coffee filter cut into 12.5 cm<sup>2</sup> triangles (Walmart, Bentonville, AR). These exposure durations were chosen to include the range of times a chemical may be held in suspension between mixing and application for a commercial field application. Most of the moisture was removed from the filter paper using a vacuum breaker. Then the coffee filter was allowed to dry before placing it in a 47 mm Petri dish sealed with parafilm.

### Evaluation of mortality

*Trichogramma ostriniae* pupae were incubated on the lab bench at room temperature (≈21 °C) for ten days and then placed in the freezer (–20 °C) to kill any emerged wasps. Twenty *E. kuehniella* eggs in each dish were chosen haphazardly and examined for emergence holes under a dissecting microscope.

## Statistical analysis

The effect of exposure time on emergence was analysed with base R software and the *drc: Analysis of Dose-Response Curves* Package in R version 4.2.1 (R Core Team, 2022; Reitz et al., 2015). The responses from the clethodim, glacial acetic acid, imazamox, and non-ionic surfactant were fitted to a Type II Weibull model (Seber & Wild, 1989):

where  $f(x)$  is the proportion of *T. ostriniae* alive at hour  $x$ ,  $c$  is the lower limit,  $d$  is the upper limit,  $e$  is the number of hours at which 50% response between the upper and lower limits occurs, the inflexion point (i.e.  $LT_{50}$ ), and  $b$  is the slope of the line at the inflexion point.

The responses from the quizalofop and copper hydroxide treatments were fitted to a two and three-parameter exponential decay model, respectively (OCED, 2006):

$$f(x) = c + (d-c)(\exp(-x/e))$$

where  $f(x)$  is the proportion of *T. ostriniae* alive at hour  $x$ ,  $c$  is the lower limit,  $d$  is the upper limit,  $e$  is the number of hours at which 50% response between the upper and lower limits occurs, the inflexion point (i.e.  $LT_{50}$ ).

For the remainder of the treatments, there was no effect of time on survival, and regression analysis was not used in the analysis. Thus, the effect of treatment on survival at 22.5-hour exposure was analysed using Proc Glimmix (SAS Institute Inc., 2019), using a beta distribution with cohort as a nested effect (Shahaba, 2012; Stroup, 2013). Following a significant treatment effect, a post hoc Dunnett-Hsu test (significance at  $P \leq 0.05$ ) was used to compare herbicide, fungicide, and adjuvant treatments with a water control.

## Results

### Effect of time

For most treatments, the duration of exposure did not affect *T. ostriniae* emergence. However, GAA (low concentration), clethodim, copper hydroxide (low concentration), imazamox (low concentration), NIS, and quizalofop did show a significant effect of duration of exposure on *T. ostriniae* survival (Table 2). The  $LT_{50}$  was between 0.8 and 37.4 h for all treatments with a significant time effect. The 22.5-h exposure will be used to assess the effect of exposure on emergence as it is the ‘worst-case’ scenario (i.e. it is unlikely that an applicator would leave a mixed solution unsprayed for longer).

### Effect of compounds

Chemical treatment had a significant effect on *T. ostriniae* emergence (Figures 1–3) ( $F = 21.49$ ;  $df = 26, 165$ ;  $P < 0.0001$ ) at 22.5 h of exposure. According to a post hoc Dunnett’s test, the air treatment was not significantly different from water (Figures 1–3). No *T. ostriniae* emerged in the quizalofop, GAA (High concentration), or  $K^+$  salt of fatty acid treatments at 22.5 h of exposure. Only two other herbicides negatively affected the emergence of *T. ostriniae* compared to water (Figure 1). The fungicide/bactericide treatments significantly reduced emergence by 25–73% depending on treatment (Figure 2). All adjuvants significantly reduced emergence except for Ammonium sulfate and both concentrations of Urea-Ammonium nitrate (Figure 3).



**Table 2.** Parameter estimates and S.E.s for the effect of exposure time on the mortality of *T. ostrinia* exposed to various adjuvants, herbicides, and fungicides.

Herbicide	Conc.	Run <sup>a</sup>	b (S.E.) <sup>b</sup>	c (S.E.)	d (S.E.)	LT <sub>50</sub> (S.E.) <sup>c</sup>
Three-parameter Weibull (Type II)						
Glacial acetic acid	104.57 g l <sup>-1</sup>	3	-0.7 (0.3)*	-	73.4 (47.9)	2.7 (4.8)
		4	-1.3 (0.4)*	-	45.5 (4.5)*	6.5 (1.2)*
Imazamox	0.37 g l <sup>-1</sup>	2	-0.6 (0.4)	-	87.0 (6.4)*	37.4 (18.2)*
		5	-0.7 (0.3)*	-	89.8 (4.0)*	35.4 (14.5)*
Four-parameter Weibull (Type II)						
Non-ionic surfactant	0.5% v v <sup>-1</sup>	2	-10.2 (81.0)	62.0 (2.9)*	96.1 (502.2)	1.5 (3.6)
		5	-2.3 (2.7)	54.2 (15.4)*	90.9 (5.3)*	7.2 (2.1)*
Clethodim	0.41 g l <sup>-1</sup>	3	-16.9 (155.9)	0.0 (1.2)	12.5 (1.5)*	5.3 (6.4)
		4	-17.9 (200.5)	0.41 (1.2)	5.0 (1.5)*	5.5 (5.5)
Two-parameter exponential decay <sup>d</sup>						
Quizalofop	0.21 g l <sup>-1</sup>	2	-	100 (-)	-	0.8 (0.1)*
		3	-	100 (-)	-	1.1 (0.1)*
Three-parameter exponential decay						
Copper hydroxide	7.2 g l <sup>-1</sup>	2	-	62.8 (3.8)	89.5 (39.5)	1.7 (1.7)
		3	-	27.1 (6.2)	78.6 (30.8)	2.9 (2.8)

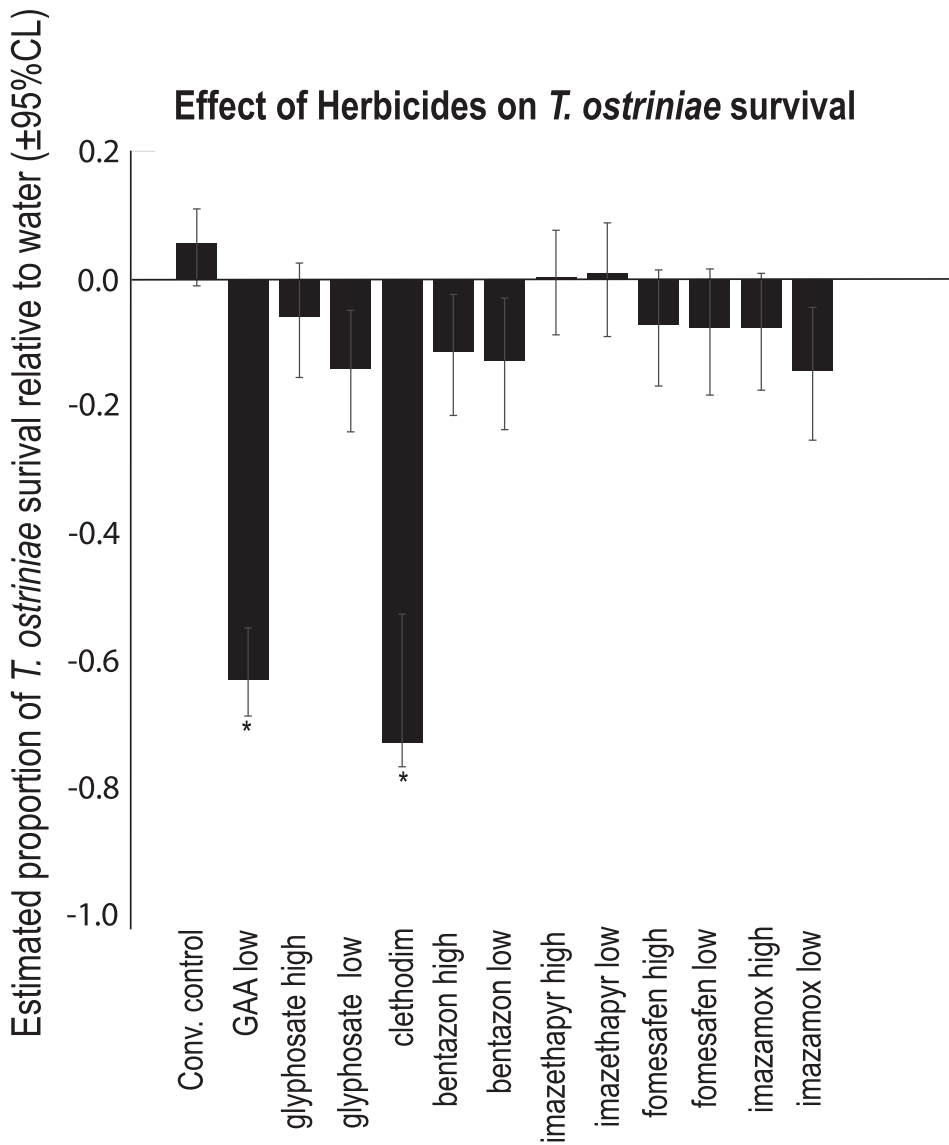
\**P*-value ≤ 0.05.<sup>a</sup>Two separate experimental runs; each run used a different *T. ostrinia* cohort.<sup>b</sup>Abbreviations – Model lacks particular parameter; b slope at LT<sub>50</sub>; c lower asymptote; d upper asymptote.<sup>c</sup>Hours.<sup>d</sup>c fixed at 100.

## Discussion

Our finding that emergence is not affected by immersion in water is similar to Gardner and Giles (1997), who found that emergence of *T. pretiosum* from eggs immersed in water ranged from about eighty to ninety per cent of an open-air control. Also similar to our findings, Khan and Ruberson (2017) found that the emulsifiable concentrate S-metolachlor negatively impacted the emergence rate of *T. pretiosum* when exposed in the pupal stage. However, the non-emulsifiable concentrates glufosinate-ammonium and nicosulfuron did not. Bastos et al. (2006) found moderately-reduced *T. pretiosum* emergence with exposure to the herbicides clomazone and a mixture of paraquat and diuron compared to water. However, Oliveira et al. (2014) did not find a decrease in the emergence of *T. galloi* with exposure to clomazone. In our study, all of the fungicides negatively affected *T. ostrinia* emergence. These findings contrast Khan and Ruberson (2017), who found that myclobutanil, pyraclostrobin, and a mixture of trifloxystrobin and tebuconazole had little effect on the emergence of *T. pretiosum*. However, the studies mentioned here only exposed *Trichogramma* pupae for 5–10 s (Bastos et al., 2006; Khan & Ruberson, 2017; Oliveira et al., 2014).

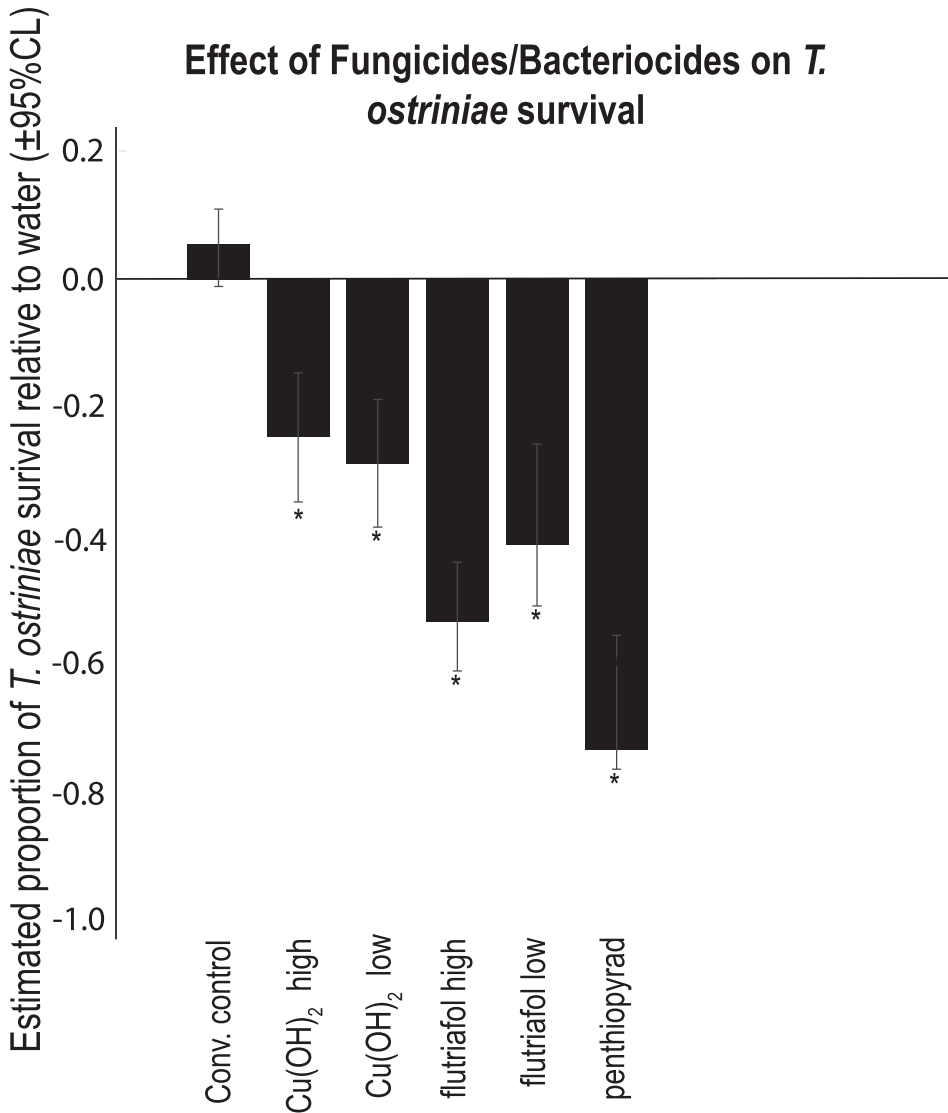
Other factors impacting parasitoid deployment by co-mixing with agrichemicals might include product formulation, parasitoid host species, and application method. All treatments tested in this study were formulated products; therefore, negative effects on emergence could be due to inert rather than active ingredients. For example, Giolo et al. (2005) found that glyphosate formulations containing isopropylamine salts were more harmful to *T. pretiosum* adults than potassium or ammonium salts. Additionally, despite being smaller, *S. cerealella* eggs have a mucus layer on the surface of the egg that *E. kuehniella* lacks. Also, the thickness of the chorion and the vitelline membrane is thicker in *S. cerealella* compared with *E. kuehniella* (Bastos et al., 2006; C nsoli





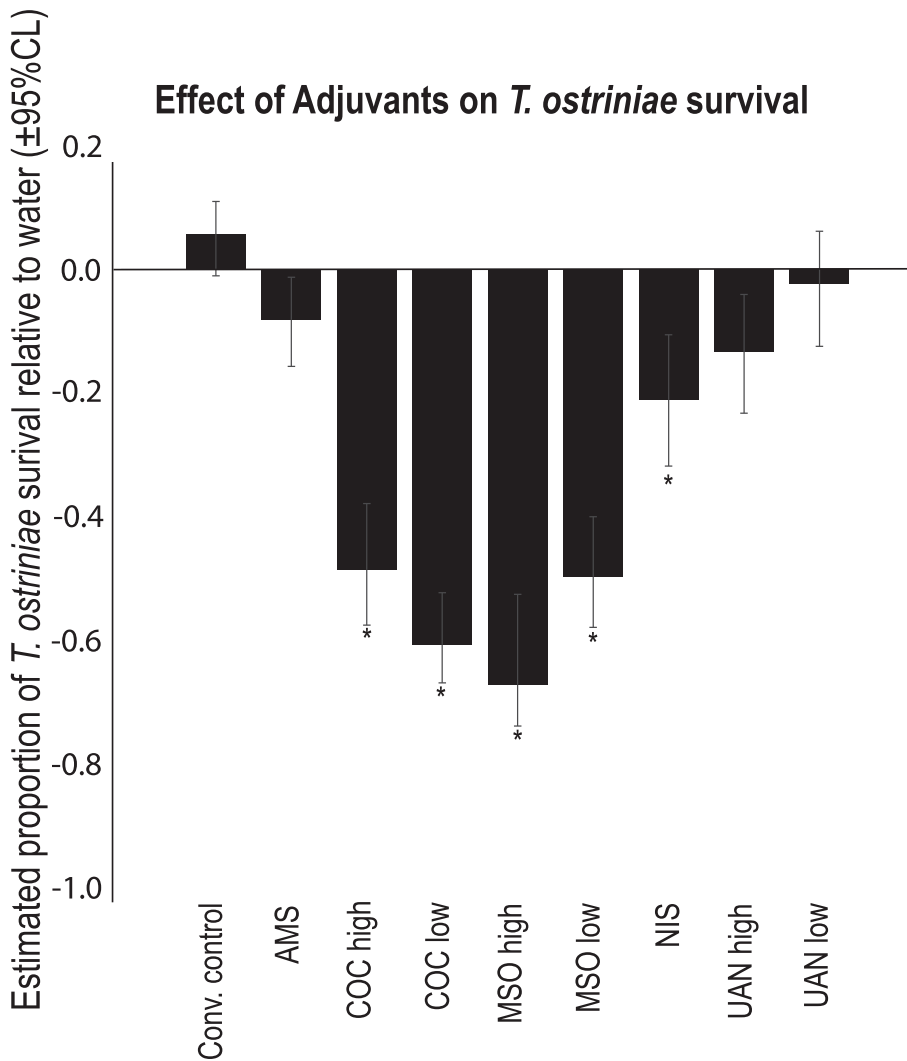
**Figure 1.** Effect of herbicides on *T. ostrinae* survival.

et al., 1999). Pesticides affect pupae developing in *S. cerealella* less than those developing in *E. kuehniella* (Bastos et al., 2006). The length of *E. kuehniella* and *S. cerealella* eggs are 520.7 and 584.7  $\mu\text{m}$ , respectively (Cônsoi et al., 1999); therefore, filter mesh size would need to accommodate the host egg size. Preliminary studies (unpublished data) indicate that a 16-mesh size would allow for the passage of *E. kuehniella* eggs. Lastly, Knutson (1996) found that passage through spray equipment reduced the emergence of an unspecified species of *Trichogramma* by about twenty per cent. However, Gardner and Giles (1997) and Gauthier and Khelifi (2016) did not find reduced emergence after the pupae were sprayed. In a study by Khelifi et al. (2019), an important source of mortality is a non-pneumatic pressure regulator. Therefore, all applicator system aspects should be



**Figure 2.** Effect of fungicides/bacteriocides on *T. ostrinae* survival.

investigated before deployment in the field. Though not examined in this study, sublethal effects of pesticides may also be a factor in biological control success. Sublethal effects include reduction in fecundity (Tai et al., 2022), longevity (Li et al., 2015; Thubru et al., 2018), perception of host cues (Bayram et al., 2010), and an increase in wing malformations (Tai et al., 2022). For instance, azadirachtin has been shown to reduce the fecundity of *Telenomus podisi* (Turchen et al., 2016), abacemtin of *Trichogramma achaeae* (Fontes et al., 2018), and essential oil of *Origanum vulgare* of *Trissolcus basal* (González et al., 2013). Wang et al. (2012) saw reduced longevity and fecundity of *Trichogramma chilonis* when exposed to fipronil and avermectins. Ribeiro et al. (2021) also saw reduced longevity and fecundity of *Trichogramma chilonis* when exposed to clothianidin. Bayram et al. (2010) found exposure to sublethal levels of



**Figure 3.** Effect of adjuvants on *T. ostriniae* survival.

cyfluthrin reduced *Telonomus busseolae* response to *Sesamia nonagrioides* pheromone. Tai et al. (2022) found increased rates of wing deformity among *Trichogramma ostriniae* individuals exposed to atrazine, difenoconazole, and propiconazole.

Even the products that reduce survival may be candidates for carriers if a broadcast release's improved effectiveness and labour savings outweigh the loss due to toxicity. A 2022 search for *Trichogramma* costs resulted in costs between \$20.00 and \$28.80 USD 100 000<sup>-1</sup> (JDC Personal observation). The U.S. minimum wage varies by state but averages \$9.80 h<sup>-1</sup> with a standard deviation of \$2.46 (Anonymous, 2022). Although, farm workers can be paid below a state's minimum wage. Furthermore, the agricultural sector is facing a labour shortage in many regions such Canada (Agriculture Canada, 2023; Komarnicki, 2012), the European Union (European Labour Authority, 2022; Mitaritonna & Ragot, 2020), India (Gunabhadra, 2017), Japan (Liu-Farrer et al., 2023),

Mexico (Zahniser et al., 2018), United Kingdom (Byrne, 2018; Scott et al., 2008; Siudek & Zawajska, 2016), and United States (Simnitt & Martin, 2022; Zahniser et al., 2018). Thus, many growers may not have time or staff to make conventional *Trichogramma* releases even if they have the financial resources.

The time stated in the literature to deploy Trichocards manually can vary from 0.5 to 17 hr ha<sup>-1</sup> (Table 3). Application by aircraft can be expensive furthermore, the high speed and altitude may cause many of the parasitoids to miss their target (Bzowska-Bakalarz et al., 2020; Zhan et al., 2021). Another option is unmanned aerial vehicles which depending on the model can treat 15 hectares in a twenty-minute flight (Zang et al., 2021). Parra and Coelho (2022) state that the labour for drone application costs \$2–3 ha<sup>-1</sup> in Brazil. However, agricultural drones can have a large upfront cost of \$10,000 or more (Nixon, 2022) which may dissuade some growers (Michles et al., 2020). Furthermore, drone usage may require a special licence and flight is limited as to where the drone may be flown (FAA, 2023; Transport Canada, 2019). Indeed, Michles et al. (2020) stated that legal barriers prevent some German growers from using drones. A further limitation of drones is limited flight duration (Martel et al., 2021). Costs associated with tractor application can be variable depending on the model and the current cost of fuel but are estimated to be \$0.86 USD ha<sup>-1</sup> for fuel, \$2.79 ha<sup>-1</sup> for repairs, and \$8.60 ha<sup>-1</sup> for depreciation (Klein & McClure, 2022). However, it should be noted that these would be incurred whether *Trichogramma* are released or not as this method takes advantage of pre-existing applications. See Table 3 for a comparison of the costs of various release methods.

In the U.S., approximately 75, 84, and 11 million acres of corn, soybean, and cotton are planted with herbicide-tolerant cultivars, respectively (Dodson, 2020; USDA-NASS, 2019). Much of this tolerance is to glyphosate. The low toxicity of glyphosate to *Trichogramma* may allow for broader adoption of spray application. For example, in cotton, glyphosate applications vary but, in some cultivars, may be made throughout much of the growing season (Beck, 2020; Wright & Munier, 2015). This may allow for control of damaging pests such as the the cotton bollworm, *Heliothis zea*, and the tobacco budworm, *H. virescens* (Bouse et al., 1980; Knutson, 1998; Stinner et al., 1974). In 2006, these pests caused over \$10 million in collective damage in Georgia alone (Roberts & Ruberson, 2007). Furthermore, the relatively low toxicity of the low concentration of copper hydroxide may allow for *Trichogramma* release with late-season disease control. Applications of copper hydroxide in dry bean and corn may be made weekly up to harvest and thus may align with the ideal time for western bean cutworm, *Striacosta albicosta*, control (Kocide, 2015; Seymour et al., 2010). It may also allow for control of European corn borer, *Ostrinia nubilalis*, in corn and potato (Anonymous, 2015; Kocide, 2015; Pavlista, 2014).

## Conclusion

This study represents the first step to proving the practicability of deploying *Trichogramma* with some commonly used pesticide formulations. Most herbicides did not affect *T. ostriniae* emergence, although most of the adjuvants and all of the fungicides tested did. Subsequent trials should assess (1) the sublethal effects of pesticides on *T. ostriniae* including possible effects on fecundity, longevity, host finding, and wing

**Table 3.** Cost (USD 100 ha<sup>-1</sup>) comparisons of release methods with a labour cost of \$9.80 hr<sup>-1</sup> taking into account increased mortality in broadcast treatments.

	Anon., 1974 as by Jones et al., 1977	Greenberg et al., 1990	Bigler, 1986	Birthal et al., 2000	Dionne et al., 2018	Greenberg et al., 1990	Dionne et al., 2018	Filippov, 1989	Current study <sup>d</sup>
Points ha <sup>-1</sup>	NS <sup>c</sup>	100	50	NS	50	∞	∞	∞	∞
Boom width (m)	–	–	–	–	–	NS	6.7	NS	12
Time (h)	80	58.3	50	1692	88.02	11.33	51.3	5.51	10.39
Labour cost (USD)	784	571.67	490	16,581.6	862.56	111.07	502.74	54.06	101.82
Total cost 0% <sup>ab</sup>	2.8	2.6	2.5	18.6	8.8	2.1	2.5	2.1	2.1
Total cost 10% <sup>b</sup>	–	–	–	–	–	2.3	2.7	2.3	2.3
Total cost 20% <sup>b</sup>	–	–	–	–	–	2.6	3.0	2.6	2.6
Total cost 30% <sup>b</sup>	–	–	–	–	–	2.7	3.1	2.6	2.7
Total cost 40% <sup>b</sup>	–	–	–	–	–	3.4	3.8	3.4	3.4
Total cost 50% <sup>b</sup>	–	–	–	–	–	4.1	4.5	4.1	4.1
Total cost 60% <sup>b</sup>	–	–	–	–	–	5.1	5.5	5.1	5.1
Total cost 70% <sup>b</sup>	–	–	–	–	–	6.8	7.2	6.7	6.8

<sup>a</sup>Assumes a release rate of 100 000 ha<sup>-1</sup> at \$20.00.

<sup>b</sup>Total cost (in thousands) cost for extra *Trichogramma* to compensate for various mortalities is as follows: 10% \$22.22 20% – \$25; 30% – \$25.57; 40% – 33.33; %50 – \$40; %60 – \$50 %70 – \$66.67 %80 – \$100 %90 – \$200.

<sup>c</sup>NS not specified.

<sup>d</sup>Hypothetical tractor calculated from UNL crop budgets.

malformations (2) the effect of the spray equipment on mortality, (3) the ability of parasitised eggs to adhere to the foliage, and (4) the level of parasitism achieved.

### Geolocation information

Nebraska, USA.

### Acknowledgements

The authors thank Kayla Mollet, Luis Ochoa, and Rick Patrick for assisting with conducting the experiment.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

### Funding

This work was funded in part by the Nebraska Dry Bean Commission (Project ID #48120) and supported by the Institute of Agriculture and Natural Resources at the University of Nebraska.

### Statements and Declarations

JDC, NCL, and JDB contributed to the design of the study. JDC and CWB conducted to day to day activities associated with the experiment. JDC produced the first draft of this manuscript with contributions from JDB and NCL. All authors commented on and approved the manuscript.

### Data availability statement

The data that support the findings of this study are available from the corresponding author, JDB, upon reasonable request.

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