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## **Effect of Duration of Deployment on Parasitism and Predation of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) Sentinel Egg Masses in Various Host Plants**

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Source: Florida Entomologist, 105(1) : 44-52

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.105.0107>

# Effect of duration of deployment on parasitism and predation of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) sentinel egg masses in various host plants

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## Abstract

The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an agricultural and nuisance pest in Georgia and Alabama, USA. Natural enemies may provide significant suppression of the brown marmorated stink bug, and sentinel egg masses are deployed commonly on plants in the field to measure their effects. The objective of this study was to evaluate the effect of deployment duration (2–5 d) on parasitism and predation of brown marmorated stink bug sentinel egg masses in plum, peach, blueberry, tomato, sassafras, corn, and soybean in these 2 states. Retrieved egg masses were processed to quantify rates of predation and parasitism and identify parasitoid species and predation types. Across crops, predation and parasitism were higher significantly in plum at 5 d compared to 2 d deployment but was similar in soybean regardless of exposure time in 2017. Predation and parasitism were higher significantly after 5 d of exposure compared to 2 d and 3 d whereas parasitism was significantly higher at 3 d compared to 2 d exposure in 2018 to 2020. For individual crop trials, though, effects of time of exposure were tested in different yr, sampling dates, and crops with variable results. When significant differences were detected for parasitism in plum, peach, tomato, and sassafras, a 5 d deployment resulted in higher parasitism compared to 2 d. Given the variability of factors that affect parasitism and predation under field conditions, we conclude that a 5 d deployment is optimal and a 3 d exposure time is minimal for assessing predation and parasitism. An additional benefit for a 5 d exposure is that it increases the probability of detecting hyperparasitism.

Key Words: brown marmorated stink bug; parasitism; predation; *Trissolcus*; *Anastatus*; *Ooencyrtus*

## Resumen

El chinche marmorino café, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), es una plaga agrícola invasora y molesta en los Estados de Georgia y Alabama, EE. UU. Los enemigos naturales pueden proporcionar una supresión significativa del chinche marmorino café y las masas de huevos centinelas se colocan comúnmente en las plantas en el campo para medir sus efectos. El objetivo de este estudio fue evaluar el efecto de la duración del despliegue (2 a 5 días) sobre el parasitismo y la depredación de las masas de huevos centinelas del chinche marmorino café en ciruela, melocotón, arándano, tomate, sasafrás, maíz y soja en estos dos Estados. Las masas de huevos recuperadas se procesaron para cuantificar las tasas de depredación y parasitismo e identificar las especies de parasitoides y los tipos de depredación. En todos los cultivos, la depredación y el parasitismo fueron significativamente más altos en ciruela a los 5 días en comparación con el despliegue de 2 días, pero fue similar en la soja independientemente del tiempo de exposición en el 2017. La depredación y el parasitismo fueron significativamente más altos después de 5 días de exposición en comparación con 2 días y 3 días, mientras que el parasitismo fue significativamente mayor también a los 3 días en comparación con la exposición a los 2 días desde el 2018 al 2020. Sin embargo, para los ensayos de cultivos individuales, los efectos del tiempo de exposición se probaron en diferentes años, fechas de muestreo y cultivos con resultados variables. Cuando se detectaron diferencias significativas para el parasitismo en ciruela, melocotón, tomate y sasafrás, el despliegue de 5 días resultó en un mayor parasitismo en comparación con 2 días. Dada la variabilidad de los factores que afectan el parasitismo y la depredación en condiciones de campo, concluimos que un despliegue de 5 días es óptimo y un tiempo de exposición de 3 días es mínimo para evaluar la depredación y el parasitismo. Un beneficio adicional de la exposición de 5 días es que aumenta la probabilidad de detectar hiperparasitismo.

Palabras Clave: chinche marmorino café; parasitismo; depredación; *Trissolcus*; *Anastatus*; *Ooencyrtus*

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive stink bug species native to China (Hoebeke & Carter 2003). Brown marmorated stink bug is an economically important agricultural pest (Leskey et al. 2012) as well as an urban nuisance (Inkley 2012). Presently, brown marmorated stink bug reproductive populations have colonized the Piedmont and are expanding into the Coastal Plain Region of Georgia and Alabama. The brown marmorated stink bug is highly polyphagous, ovipositing on a variety of hosts (Bakken et al. 2015; Bergmann et al. 2016), including sassafras (*Sassafras albidum* [Nutt.] Nees; Lauraceae), a non-crop host that is found in woodlands bordering agricultural fields in this region. In the southeastern US, populations of brown marmorated stink bug feed on and oviposit in fruit, vegetable, and row crops. Natural enemies (i.e., predators and parasitoids) may provide effective means of controlling brown marmorated stink bugs in this region, and sentinel egg masses often are deployed to assess potential suppression (Cornelius et al. 2016a, b; Herlihy et al. 2016; Morrison et al. 2016; Ogburn et al. 2016; Dieckhoff et al. 2017; Tillman et al. 2020). However, the duration of egg mass deployment may affect parasitism and predation rate. Therefore, it is crucial for assessing biological control services to understand the relative effects of sentinel egg deployment duration in a variety of hosts on the responses of native parasitoids and predators to this invasive pest.

Field surveys on biological control of brown marmorated stink bug eggs in various agroecosystems in the US have revealed that native natural enemies attack egg masses at varying rates of parasitism and predation. Presently, 20 species of Nearctic hymenopteran primary endoparasitoids in the genera *Anastatus* Motschulsky (Eupelmidae), *Trissolcus* Ashmead (Scelionidae), *Telenomus* Haliday (Scelionidae), *Gryon* Haliday (Scelionidae), and *Ooencyrtus* Ashmead (Encyrtidae) (all Hymenoptera) have been reported to parasitize brown marmorated stink bug eggs (Cornelius et al. 2016a, b; Herlihy et al. 2016; Morrison et al. 2016; Ogburn et al. 2016; Dieckhoff et al. 2017; Jones et al. 2017; Balusu et al. 2019a, b; Tillman et al. 2020). Adventive populations of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), a parasitoid of brown marmorated stink bug in its native range, have been found in several US states but not the southeast (Talamas et al. 2015a; Milnes et al. 2016). A diverse complex of chewing and piercing-sucking predators attack brown marmorated stink bug eggs. Morrison et al. (2016) categorized predators of brown marmorated stink bug eggs into 4 characteristic patterns of predator feeding damage: complete chewing by Gryllidae and Tettigoniidae; incomplete chewing by Coccinellidae, Carabidae, and Dermaptera; stylet sucking by Anthocoridae and Pentatomidae; and punctured sucking by Salticidae. In addition, Tillman et al. (2020) described removal of whole eggs from egg masses by Formicidae, hole sucking by late instar *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and non-stylet sucking by *Nabis* spp. La-treille (Hemiptera: Nabidae).

The method by which brown marmorated stink bug egg mass predation and parasitism is measured may yield different patterns of

activity. Higher rates of parasitism have been found in wild (naturally oviposited) brown marmorated stink bug eggs compared to fresh sentinel (laboratory-reared) eggs (Jones et al. 2014; Dieckhoff et al. 2017). Wild brown marmorated stink bug eggs may be attacked by a greater number of parasitoid species compared to sentinel egg masses (Jones et al. 2014), or the number of undeveloped parasitoids may differ in wild versus fresh sentinel eggs (Dieckhoff et al. 2017). Using wild egg masses to assess parasitism and predation is beneficial because the timing of assessment is linked to the phenology of both brown marmorated stink bug and host plants in the landscape. However, wild eggs can be challenging to find on host plants, especially in sufficient quantities for assessing rates of parasitism and predation. As such, deploying sentinel egg masses on host plants is necessary to assess patterns of parasitism and predation of brown marmorated stink bug egg masses across hosts and locations. Moreover, in recent stink bug sentinel egg studies, the number of d egg masses were deployed in fields ranged from 1 to 5 d (Cornelius et al. 2016a, b; Herlihy et al. 2016; Morrison et al. 2016; Ogburn et al. 2016; Dieckhoff et al. 2017; Jones et al. 2017; Tillman et al. 2020), which likely affects observed amounts of predation and parasitism. Therefore, determining the amount of time sentinel egg masses should be deployed for any given host is necessary to compare between and interpret patterns of brown marmorated stink bug egg predation and parasitism.

The objective of this study was to test the effect of deployment duration on predation and parasitism of brown marmorated stink bug sentinel egg predation and parasitism by native natural enemies in a variety of hosts. Over a 4-yr period, retrieved sentinel egg masses were evaluated for parasitism and predation at 2 to 5 d of exposure in plum, peach, blueberry, tomato, sassafras, corn, and soybean in Georgia and Alabama.

## Materials and Methods

This study was conducted at 6 sites in Georgia and Alabama (Table 1). The presence of wild brown marmorated stink bug populations were confirmed at each site. Insecticides were not applied to plant species during the season. Brown marmorated stink bug sentinel egg masses were deployed in plum, peach, blueberry, tomato, sassafras, corn, and soybean.

### BROWN MARMORATED STINK BUG SENTINEL EGG MASSES

Egg masses came from a brown marmorated stink bug colony reared in cages (27.9 cm long × 26.7 cm wide × 20.3 cm tall) on whole bean pods, apple slices, and raw peanuts at the USDA, ARS Crop Protection & Management Research Laboratory in Tifton, Georgia, USA. Knit cloth (97% cotton, 3% spandex) (JoAnn Stores, LLC, Hudson, Ohio, USA) was used as a substrate for oviposition (Tillman et al. 2020). In

**Table 1.** Sites, locations, and host plants where brown marmorated stink bug sentinel egg masses were deployed.

Site	Latitude, longitude	Host plant <sup>a</sup>
Auburn University, Auburn, Alabama, USA	32.5913°N, 85.4935°W	SO
Chilton Research & Extension Center, Clanton, Alabama, USA	32.9199°N, 86.6720°W	PE
Foster Brady Farm, Monroe, Georgia, USA	33.8345°N, 83.5822°W	TO
Prattville Agricultural Research Unit, Prattville, Alabama, USA	32.4286°N, 86.4458°W	CO, SO, SA
University of Georgia Bledsoe Research Farm, Williamson, Georgia, USA	33.1769°N, 84.4076°W	SO
University of Georgia Horticulture Research Farm, Watkinsville, Georgia, USA	33.8835°N, 83.4203°W	BB
USDA, ARS, Fruit & Tree Nut Research Laboratory, Byron, Georgia, USA	32.3910°N, 83.4321°W	PL

<sup>a</sup>SO = soybean; PE = peach; TO = tomato; CO = corn; SA = sassafras; PL = plum; BB = blueberry.

general, there are 28 eggs per egg mass for brown marmorated stink bugs (Nielsen & Hamilton 2009), but in this study any damaged eggs were gently removed from an egg mass when eggs were counted; thus, the mean number of eggs per egg masses was  $26.51 \pm 2.82$  SD. In 2017, frozen egg masses ( $\leq 12$  h old when placed in a freezer at  $-20$  °C for 1–4 d) were deployed. In 2018 and 2019, refrigerated egg masses ( $\leq 12$  h old when placed in a refrigerator with a temperature range of 2.8 to 3.3 °C for 24 h) were used. Unlike frozen egg masses, refrigerated eggs retain typical shape and color when deployed in field. Refrigerated eggs do not hatch; therefore first instars do not feed on eggs in the field, which sometimes occurs with fresh egg masses in the laboratory. In the field, sentinel egg masses were suspended on stems or branches at mid-height of plants using paperclips. In 2020, parasitoid species ovipositing in brown marmorated stink bug eggs on sassafras were observed and recorded for approximately 1 to 4 h immediately after deployment. Retrieved egg masses were held in a walk-in environmental chamber ( $25$  °C  $\pm 2.0$  °C;  $50 \pm 10\%$  RH; 12:12 h [L:D] photoperiod) usually for 12 to 14 d for parasitoid emergence.

## EXPERIMENTAL TREATMENTS

For timed exposure trials, 6 to 20 brown marmorated stink bug sentinel egg masses (average of 15) were deployed for each treatment in a host plant. Note that hereafter we refer to a trial in a specific host plant on a specific date as a round. Taylor et al. (2014) reported that brown marmorated stink bug neonates emerge 3 to 6 d after oviposition in laboratory; however, natural egg masses in field in the southeast US emerge around 6 d after oviposition (G. Tillman, unpublished data). Thus, the maximum amount of exposure time was 5 d. In 2017, paired sentinel egg masses were exposed to natural enemies for 2 and 5 d. For this yr, egg masses were deployed in plum in Byron, Georgia, USA, on 14 Jun, 19 Jul, 16 Aug, 20 Sep, and 18 Oct; and in soybean in Williamson, Georgia, USA, on 7, 19, and 23 Sep. In 2018 and 2019, sentinel egg masses were deployed for 2, 3, and 5 d. In 2018, egg masses were deployed in plum in Byron, Georgia, on 22 May, 10 Jul, and 7 Aug; in blueberry in Watkinsville, Georgia, USA, on 19 Jun; and in soybean in Auburn, Alabama, USA, on 6 Aug and in Prattville, Alabama, USA, on 7 Aug. In 2019, egg masses were deployed in plum in Byron, Georgia, on 5 and 23 Jun; in peach in Clanton, Alabama, USA, on 5 and 16 Jul; in blueberry in Watkinsville, Georgia, on 17 Jun; in tomato in Monroe, Georgia, USA, on 27 Jun; in sassafras in Prattville, Alabama, on 8 Jul; in corn in Prattville, Alabama, on 6 and 16 Jul; and in soybean in Prattville, Alabama, on 7 and 21 Aug. In 2020, a study was conducted in sassafras in Prattville, Alabama, on 16 Aug to examine deployment at 3, 4, and 5 d.

## PARASITISM AND PREDATION OF EGG MASSES

Retrieved egg masses were processed to quantify the rate of predation and parasitism as well as identify parasitoid species and types of predation on egg masses. As needed, eggs were dissected for dead immature parasitoids. Determination of immatures to parasitoid family was based on the following: differentiating ovipositional marks of *Ooencyrtus*, *Anastatus*, and *Trissolcus* on eggs (Tillman et al. 2020), descriptions of immature *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) in Volkoff and Colazza (1992), and photographs of immature *Anastatus redivii* (Howard) (Hymenoptera: Eupelmidae) in brown marmorated stink bug eggs (P. G. Tillman, unpublished). For adults, *Trissolcus* species were identified using the Talamas et al. (2015b) key; *Anastatus* species were identified using the Burks (1967) key; *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) was identified using the Johnson (1984) key. Predation was divided into 7 feeding categories:

(1) complete chewing, (2) incomplete chewing, (3) stylet sucking, (4), punctured sucking, (5) taken eggs, (6) hole sucking, and (7) non-stylet sucking (see Tillman et al. 2020 for descriptions and photographs of each category). Voucher specimens of parasitoid species are deposited in the Florida State Collection of Arthropods, Gainesville, Florida, USA.

## STATISTICAL ANALYSES

Predation and parasitism of sentinel brown marmorated stink bug eggs were analyzed using SAS statistical software (SAS 2012). Because egg masses were collected on d 2 and 5 in 2017, we analyzed this yr separately. To test whether exposure time affected percent predation and parasitism of egg masses across crops, data for all crops were analyzed with generalized linear mixed models (PROC GLIMMIX; SAS 2012) using a beta distribution. Fixed effects included crop type, exposure time, and a 2-way interaction between crop type and exposure time. Random effects included yr and round. For each model, we tested whether the 2-way interaction between crop type and exposure time significantly improved model fit. The crop type by exposure time interaction was significant for the 2017 model. However, it did not improve model fit for the 2018 to 2020 model and, therefore, was dropped from the final model. Treatment means were compared using an *F*-test and means were further separated using Tukey's honestly significant difference (HSD). In SAS, we back transformed means for presentation in tables (ILINK option).

To test whether exposure time affected percent predation and parasitism of egg masses for individual rounds of crops, the 2017 data were compared between the 2 and 5 d exposure times using paired *t*-tests for each round. In 2018 to 2020, percent predation and parasitism of egg masses were analyzed with generalized linear mixed models (PROC GLIMMIX; SAS 2012) using a beta distribution. Exposure time (2, 3, or 5 d) was included as a fixed effect. Treatment means were compared using an *F*-test and means were further separated using Tukey's honestly significant difference (HSD). Means were back-transformed for presentation in tables (ILINK option).

## Results

### PREDATION

Across crops in 2017, percent predation of brown marmorated stink bug sentinel egg masses was predicted by the interaction between crop type and duration of deployment ( $F_{3,155} = 3.14$ ;  $P = 0.0267$ ) of brown marmorated stink bug sentinel egg masses. Percent predation was significantly higher in plum exposed for 5 d compared to 2 d, whereas in soybean percent predation was similar regardless of exposure time (Table 2). Predation was significantly lower in plum at the 2 d deployment time than for soybean at either 2 d or 5 d of exposure.

In 2018 to 2020, percent of predation of brown marmorated stink bug sentinel egg masses was predicted by duration of deployment ( $F_{2,699} = 4.09$ ;  $P = 0.017$ ) and crop type ( $F_{6,699} = 2.74$ ;  $P = 0.012$ ). Regardless of crop type, percent predation was significantly higher after 5 d of exposure in the field compared to the 2 d and 3 d exposure times (Table 2). Percent predation levels were the lowest in tomato followed by blueberry, and highest in peach, corn, plum, and sassafras.

In plum, comparison of exposure time within rounds suggests that percent predation of brown marmorated stink bug sentinel egg masses varied by round and within the season. In 2017, significantly higher percent predation was detected for the 5 d deployment treatment compared to 2 d for round 3 (Table 3). Percent predation of brown

**Table 2.** Means ( $\pm$  SE) for percent predation and parasitism of brown marmorated stink bug sentinel eggs by crop and duration of deployment from 2017 to 2020.

Yr	Crop	D	% Predation	% Parasitism
2017	Plum	2	24.9 $\pm$ 6.3 b	27.8 $\pm$ 9.3 b
		5	38.9 $\pm$ 7.7 a	48.7 $\pm$ 8.7 a
	Soybean	2	45.9 $\pm$ 6.5 a	9.5 $\pm$ 7.4 b
		5	43.8 $\pm$ 6.4 a	12.5 $\pm$ 6.9 b
2018 to 2020	All	2	21.7 $\pm$ 6.7 b	0.8 $\pm$ 0.5 c
		3	21.4 $\pm$ 6.7 b	1.48 $\pm$ 0.8 b
		5	29.4 $\pm$ 8.1 a	2.58 $\pm$ 1.4 a
	Peach	All	39.9 $\pm$ 11.4 a	0.7 $\pm$ 0.5 c
	Corn	All	29.9 $\pm$ 9.7 ab	1.6 $\pm$ 1.0 bc
	Plum	All	28.5 $\pm$ 6.6 ab	0.8 $\pm$ 0.4 c
	Sassafras	All	28.1 $\pm$ 9.6 ab	3.8 $\pm$ 2.3 a
	Soybean	All	23.9 $\pm$ 6.7 b	1.3 $\pm$ 0.7 bc
	Blueberry	All	21.9 $\pm$ 6.0 bc	1.1 $\pm$ 0.6 bc
	Tomato	All	7.4 $\pm$ 4.9 c	2.7 $\pm$ 1.7 ab

In 2017, for each crop by duration of deployment, means followed by the same letter in the same column are not significantly different (Tukey's HSD,  $\mu = 0.05$ ). In 2018 to 2020, for each duration of deployment and also for each crop, means followed by the same letter in the same column are not significantly different (Tukey's HSD,  $\mu = 0.05$ ).

marmorated stink bug egg masses was influenced significantly by the deployment duration in this fruit crop in 2018 for round 2 and in 2019 for both rounds (Table 4). In each case, percent predation was higher after a 5 d deployment time than after only 2 d (Tables 5, 6). For round 1 of 2019, the rate of predation was higher for the 3 d exposure time compared to 2 d. In general, percent predation increased in plum over the season through Sep (Tables 3, 5, 6). Early in the season, complete (Acrididae, Tettigoniidae, and Gryllidae) and incomplete (Coccinellidae larvae) chewing predators and removal of egg masses (Formicidae, especially *Solenopsis invicta* Buren [Hymenoptera: Formicidae]) were prevalent. However, punctured sucking predation (Salticidae) and piercing-sucking hole (Chrysopidae, mainly *Chrysoperla carnea* [Stephens] [Neuroptera: Chrysopidae]) predation also were detected. By

mid-season, stylet piercing-sucking predation was detected, primarily due to cannibalism by brown marmorated stink bug nymphs.

In soybean, comparison of exposure time within rounds suggests that percent predation of brown marmorated stink bug sentinel egg masses also varied by round in this row crop. Percent predation was significantly higher at 5 d than 2 d deployment for round 2 in 2017 (Table 3), and for round 1 in 2018 where predation was higher at 3 d deployment compared to 5 d (Tables 4, 5). Similar types of predation, mainly complete chewing (Acrididae, Tettigoniidae, and Gryllidae) and removal of egg masses (Formicidae, especially *S. invicta*) and some stylet sucking (Anthocoridae, primarily *Orius insidiosus* [Say] [Hemiptera: Anthocoridae], and Geocoridae, primarily *Geocoris punctipes* [Say] [Hemiptera: Geocoridae]) were detected in all soybean plots over time.

In contrast, in peach, blueberry, tomato, corn, and sassafras, deployment duration did not affect predation of sentinel egg masses by round (Tables 4, 5, 6). In peach, stylet piercing-sucking predation was the dominant type of predation detected upon examination of eggs in the laboratory. Chewing and stylet piercing-sucking predation, as well as some punctured sucking predation, were detected for egg masses deployed in blueberry. In tomato, complete chewing predation (Acrididae and Tettigoniidae) was the prevailing type of feeding damage on egg masses. In corn, complete chewing, egg removal, and stylet-sucking predation were detected for the brown marmorated stink bug sentinel egg masses. In sassafras, complete and incomplete chewing, punctured and stylet piercing-sucking, and removal of eggs from egg masses were the main types of predation on brown marmorated stink bug eggs.

#### PARASITISM

Across crops in 2017, percent parasitism of brown marmorated stink bug sentinel egg masses was predicted by a significant interaction between deployment duration and crop type ( $F_{3,155} = 4.89$ ;  $P = 0.004$ ). Similar to patterns of predation, percent parasitism was significantly higher for 5 d of exposure compared to 2 d in plum and similar in soybean at each of these exposure times (Table 2). Parasitism was significantly higher for plum at the 5 d exposure time than for soybean at either deployment time.

**Table 3.** Means ( $\pm$  SE) for percent predation and parasitism of brown marmorated stink bug sentinel eggs 2 and 5 d after exposure to plum in Byron, Georgia, USA, and soybean in Williamson, Georgia, USA, in 2017.

Crop	Round	Date	D	% Predation				% Parasitism			
				Mean	t	df	P	Mean	t	df	P
Plum	1	14 Jun	2	14.6 $\pm$ 8.8 a	0.53	5	0.621	6.9 $\pm$ 6.9 a	2.20	5	0.079
			5	8.8 $\pm$ 3.5 a				45.2 $\pm$ 19.9 a			
	2	19 Jul	2	16.0 $\pm$ 8.9 a	1.13	11	0.284	1.6 $\pm$ 1.6 a	1.63	11	0.131
			5	27.6 $\pm$ 11.0 a				12.6 $\pm$ 7.6 a			
	3	16 Aug	2	5.8 $\pm$ 2.8 b	3.6	29	0.001	3.8 $\pm$ 2.9 a	1.38	29	0.177
			5	31.1 $\pm$ 7.2 a				12.3 $\pm$ 5.3 a			
	4	20 Sep	2	20.3 $\pm$ 4.9 a	1.99	29	0.056	6.2 $\pm$ 3.8 a	1.88	29	0.070
			5	38.4 $\pm$ 7.4 a				17.5 $\pm$ 5.5 a			
	5	18 Oct	2	4.7 $\pm$ 1.6 a	1.50	29	0.145	1.9 $\pm$ 1.8 b	2.27	29	0.031
			5	10.4 $\pm$ 7.4 a				16.4 $\pm$ 5.9 a			
Soybean	1	7 Sep	2	66.5 $\pm$ 8.1 a	1.45	28	0.158	1.1 $\pm$ 0.7 a	0.79	28	0.436
			5	77.7 $\pm$ 6.7 a				2.1 $\pm$ 1.1 a			
	2	19 Sep	2	51.3 $\pm$ 0.9 b	2.54	29	0.017	0.7 $\pm$ 0.4 a	0.54	29	0.593
			5	73.1 $\pm$ 7.1 a				1.1 $\pm$ 0.6 a			
	3	23 Sep	2	34.4 $\pm$ 0.8 a	0.79	28	0.437	0.1 $\pm$ 0.4 a	1.36	28	0.184
			5	43.0 $\pm$ 7.5 a				0.4 $\pm$ 0.6 a			

For each round of deployment by crop, means followed by the same letter in the same column are not significantly different (paired t-test,  $\mu = 0.05$ ).

**Table 4.** Fixed effect estimates of the influence of exposure time on percent predation and parasitism of brown marmorated stink bug sentinel eggs in host plants in 2018, 2019, and 2020.

Crop	Yr	Round	Date	% Predation			% Parasitism		
				F	df	P	F	df	P
Plum	2018	1	22 May	0.89	2, 57	0.417	3.89	2, 57	0.026
		2	10 Jul	4.41	2, 57	0.017	1.65	2, 57	0.201
		3	7 Aug	2.23	2, 57	0.117	0.15	2, 57	0.862
	2019	1	5 Jun	5.79	2, 27	0.008	1.03	2, 27	0.384
		2	23 Jun	3.25	2, 42	0.049	1.67	2, 42	0.200
Blueberry	2018	1	19 Jun	0.15	2, 24	0.859	2.11	2, 24	0.164
	2019	1	17 Jun	1.10	2, 32	0.344	0.24	2, 32	0.791
Peach	2019	1	5 Jul	0.64	2, 42	0.538	0.26	2, 42	0.769
Peach		2	16 Jul	0.19	2, 27	0.831	7.79	2, 27	0.006
Tomato	2019	1	27 Jun	1.31	2, 42	0.280	13.5	2, 42	0.0001
Sassafras	2019	1	8 Jul	1.63	2, 42	0.208	6.36	2, 42	0.004
	2020	2	16 Aug	0.22	2, 51	0.802	7.34	2, 51	0.002
Corn	2019	1	6 Jul	0.22	2, 42	0.800	0.93	2, 42	0.402
		2	16 Jul	0.51	2, 42	0.604	0.87	2, 42	0.428
Soybean	2018	1	6 Aug	3.26	2, 57	0.046	0.01	2, 57	0.992
		2	7 Aug	0.52	2, 54	0.598	0.08	2, 54	0.923
	2019	1	7 Aug	0.14	2, 42	0.867	0.30	2, 42	0.74
		2	21 Aug	1.63	2, 42	0.207	0.89	2, 42	0.417

Analyses used PROC GLIMMIX model with a beta distribution.

Across crops in 2018 to 2020, the percent of parasitism of brown marmorated stink bug sentinel egg masses was significantly influenced by duration of deployment ( $F_{2,858} = 10.48$ ;  $P < 0.0001$ ) and crop type ( $F_{6,858} = 5.58$ ;  $P < 0.0001$ ). Percent parasitism was significantly higher after 5 d of exposure in the field compared to a 3 d exposure time and higher after 3 d of exposure compared to 2 d (Table 2). Percent parasitism was highest in sassafras compared to all other host plants except tomato (Table 2). Parasitism was very low in peach and plum.

In plum in 2017, percent parasitism of brown marmorated stink bug sentinel egg masses was significantly higher when deployed for 5 d compared to 2 d for round 5 (Table 3). It was numerically higher at 5 d than 2 d in round 1 (only 6 egg masses) and 4; at the time of each of these rounds, predation was relatively low. Percent parasitism of brown marmorated stink bug egg masses was influenced significantly by the length of time the egg masses were deployed in this fruit crop in round 1 in 2018 (Table 4), and was higher when eggs were deployed for

**Table 5.** Least squares mean ( $\pm$  SE) for percent predation and parasitism of brown marmorated stink bug sentinel eggs 2, 3, and 5 d after exposure to host plants in 2018.

Site	Crop	Round	Date	D	% Predation	% Parasitism
Byron, Georgia, USA	Plum	1	22 May	2	8.4 $\pm$ 2.6 a	9.7 $\pm$ 2.9 b
				3	5.6 $\pm$ 1.8 a	2.6 $\pm$ 2.5 b
				5	6.4 $\pm$ 2.1 a	19.1 $\pm$ 0.5 a
		2	10 Jul	2	21.7 $\pm$ 5.2 b	5.0 $\pm$ 1.8 a
				3	16.8 $\pm$ 4.2 b	7.6 $\pm$ 2.6 a
				5	40.2 $\pm$ 7.4 a	4.4 $\pm$ 1.6 a
		3	7 Aug	2	62.9 $\pm$ 7.2 a	1.6 $\pm$ 0.7 a
				3	72.2 $\pm$ 6.1 a	1.9 $\pm$ 0.8 a
				5	80.2 $\pm$ 4.8 a	1.7 $\pm$ 0.7 a
Watkinsville, Georgia, USA	Blueberry	1	19 Jun	2	33.38 $\pm$ 9.8 a	7.9 $\pm$ 3.7 a
				3	26.47 $\pm$ 8.2 a	9.6 $\pm$ 4.2 a
				5	30.14 $\pm$ 7.3 a	18.3 $\pm$ 3.4 a
Auburn, Alabama, USA	Soybean	1	6 Aug	2	46.1 $\pm$ 7.7 b	2.0 $\pm$ 0.8 a
				3	72.1 $\pm$ 6.1 a	2.0 $\pm$ 0.8 a
				5	64.9 $\pm$ 7.3 ab	2.0 $\pm$ 0.8 a
Prattville, Alabama, USA		2	7 Aug	2	62.3 $\pm$ 7.6 a	3.6 $\pm$ 1.4 a
				3	70.9 $\pm$ 6.3 a	3.3 $\pm$ 1.3 a
				5	71.9 $\pm$ 6.3 a	3.8 $\pm$ 1.5 a

For each effect, least squares means followed by the same letter in the same column are not significantly different (Tukey's HSD,  $\mu = 0.05$ ).

**Table 6.** Least squares mean ( $\pm$  SE) for percent predation and parasitism of brown marmorated stink bug sentinel eggs 2, 3, and 5 d after exposure in host plants in 2019, and 3, 4, and 5 d after exposure to sassafras (Round 2) in 2020.

Site	Crop	Round	Date	D	% Predation	% Parasitism
Byron, Georgia, USA	Plum	1	5 Jun	2	15.5 $\pm$ 4.1 b	12.8 $\pm$ 6.5 a
				3	31.9 $\pm$ 6.0 a	17.6 $\pm$ 7.5 a
				5	44.3 $\pm$ 7.9 a	26.7 $\pm$ 7.8 a
		2	23 Jun	2	77.5 $\pm$ 6.3 b	0.1 $\pm$ 0.1 a
				3	83.0 $\pm$ 5.1 ab	0.3 $\pm$ 0.1 a
				5	90.7 $\pm$ 7.5 a	0.3 $\pm$ 0.1 a
Clanton, Alabama, USA	Peach	1	5 Jul	2	34.2 $\pm$ 11.0 a	4.2 $\pm$ 1.8 a
				3	30.4 $\pm$ 11.0 a	5.4 $\pm$ 2.2 a
				5	50.2 $\pm$ 14.0 a	5.4 $\pm$ 2.2 a
Clanton, Alabama, USA	Peach	2	16 Jul	2	37.8 $\pm$ 10.4 a	15.5 $\pm$ 6.7 b
				3	29.3 $\pm$ 9.8 a	6.8 $\pm$ 3.3 b
				5	33.9 $\pm$ 13.8 a	52.0 $\pm$ 10.9 a
Watkinsville, Georgia, USA	Blueberry	1	17 Jun	2	8.4 $\pm$ 2.8 a	6.9 $\pm$ 3.1 a
				3	12.0 $\pm$ 3.9 a	8.5 $\pm$ 3.8 a
				5	14.4 $\pm$ 7.3 a	9.2 $\pm$ 3.9 a
Monroe, Georgia, USA	Tomato	1	27 Jun	2	17.5 $\pm$ 5.3 a	5.2 $\pm$ 1.8 c
				3	14.1 $\pm$ 4.4 a	12.4 $\pm$ 3.8 b
				5	25.0 $\pm$ 6.9 a	35.1 $\pm$ 7.5 a
Prattville, Alabama, USA	Sassafras	1	8 Jul	2	29.4 $\pm$ 7.2 a	4.5 $\pm$ 1.7 b
				3	49.7 $\pm$ 8.9 a	7.4 $\pm$ 2.8 b
				5	44.2 $\pm$ 9.1 a	17.0 $\pm$ 5.6 a
		2	16 Aug	3	13.6 $\pm$ 4.0 a	30.9 $\pm$ 6.4 b
				4	16.7 $\pm$ 4.6 a	64.1 $\pm$ 6.9 a
				5	16.3 $\pm$ 4.6 a	68.4 $\pm$ 6.5 a
Prattville, Alabama, USA	Corn	1	6 Jul	2	40.5 $\pm$ 8.7 a	13.2 $\pm$ 4.2 a
				3	33.2 $\pm$ 7.9 a	21.3 $\pm$ 6.3 a
				5	38.7 $\pm$ 8.6 a	18.0 $\pm$ 5.4 a
		2	16 Jul	2	18.4 $\pm$ 5.4 a	16.1 $\pm$ 4.9 a
				3	14.2 $\pm$ 4.4 a	11.9 $\pm$ 3.8 a
				5	19.9 $\pm$ 5.8 a	18.9 $\pm$ 5.5 a
Prattville, Alabama, USA	Soybean	1	7 Aug	2	5.8 $\pm$ 2.3 a	7.9 $\pm$ 3.0 a
				3	5.7 $\pm$ 2.3 a	7.8 $\pm$ 2.9 a
				5	6.8 $\pm$ 2.7 a	10.6 $\pm$ 3.7 a
		2	21 Aug	2	22.8 $\pm$ 6.4 a	15.6 $\pm$ 4.6 a
				3	14.7 $\pm$ 4.5 a	20.9 $\pm$ 5.9 a
				5	12.1 $\pm$ 3.9 a	24.7 $\pm$ 6.6 a

For each effect, least squares means followed by the same letter in the same column are not significantly different (Tukey's HSD,  $\mu = 0.05$ ).

5 d than for 2 d and 3 d (Table 5). Parasitism was numerically higher for the 5 d exposure time than for the shorter deployment times for round 1 in 2019 (Table 6). In 2018, the diversity of parasitoid species, *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae), *Trissolcus brochymenae* (Ashmead) (Hymenoptera: Scelionidae), *Trissolcus solocis* Johnson (Hymenoptera: Scelionidae), *Te. podisi*, *An. redivii*, and *Ooencyrtus* sp., that emerged from brown marmorated stink bug sentinel egg masses was higher than in 2017 and 2019 where only *Tr. euschisti*, *An. redivii*, and *Ooencyrtus* sp. emerged from sentinel egg masses.

In peach, although deployment duration did not significantly affect parasitism of sentinel egg masses in early Jul 2019 (round 1) (Table 4), during round 2 exposure time did significantly affect parasitism (Tables 4, 6). The rate of parasitism was higher when the exposure time was extended to 5 d (Table 6). Mainly *Ooencyrtus* sp. emerged from egg masses in peach, but *Te. podisi* and *An. redivii* also parasitized eggs in this host crop.

In tomato, percent parasitism was influenced by exposure time in Jun 2019 (Table 4). Unlike peach, percent parasitism was highest for

the 5 d exposure than the other 2 exposure times, and 3 d was higher than 2 d exposure (Table 6). Eggs were parasitized primarily by *Gyron obesum* Masner (Hymenoptera: Scelionidae), but also *Tr. brochymenae* and *Ooencyrtus* sp. in tomato.

In sassafras, percent parasitism was affected by exposure time in 2019 and 2020 (Table 4). In 2019, percent parasitism was higher at 5 d deployment compared to the 2 d and 3 d treatments (Table 6). In 2020, 5 d deployment resulted in higher percent parasitism compared to the 3 d treatment, but percentages were similar between 4 and 5 d of exposure. Also, 16.7% of egg masses were 100% parasitized at 4 and 5 d whereas 11.1% of those parasitized on d 2 of exposure were completely parasitized. Interestingly, approximately 1 to 4 h after deployment in sassafras in 2020, 8 of the egg masses were observed with an ovipositing *T. euschisti* female, and 5 of them were observed with an ovipositing *An. redivii* female. Over both yr, retrieved egg masses were parasitized by *Tr. euschisti*, *An. redivii*, *Ooencyrtus* sp., *Tr. brochymenae*, and *Te. podisi*.

In blueberry, deployment duration did not affect parasitism of sentinel egg masses in 2018 and 2019 even though parasitism was numeri-

cally higher at 5 d than at the 2 d and 3 d exposure times in 2018 (Table 4, 5, 6). The only parasitoid species emerging from brown marmorated stink bug egg masses was *An. reduvii* and *Tr. brochymenae* in 2018 and 2019, respectively.

In soybean and corn, duration of deployment did not significantly affect percent parasitism (Tables 3, 4, 5, 6). The diversity of parasitoid species emerging from brown marmorated stink bug eggs in soybean was relatively low, consisting primarily of *Ooencyrtus* sp. and few *Te. podisi*, with the exception of *Tr. basalis* that parasitized brown marmorated stink bug eggs when the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), also was present in soybean. In corn, brown marmorated stink bug eggs were parasitized by *Ooencyrtus* sp., *Te. podisi*, and *Tr. basalis*.

## Discussion

Across crops, overall patterns regarding the influence of duration of exposure on percent predation and parasitism of brown marmorated stink bug egg masses in the field were detected. Indeed, deployment at 5 d increased the rate of predation beyond that for the 2 d and 3 d deployment time. Unlike percent predation, the rate of parasitism also was higher for 3 d compared to 2 d. Thus, perhaps 5 d is the optimal deployment time to assess percent predation and parasitism.

For the individual within-crop rounds, the effects of time of exposure were tested in different yr, sampling dates, and different crops with variable results. Although deployment time significantly increased predation and parasitism rates for some rounds, in others it had no significant effect. For example, for the 2 crops in 2017, exposure time significantly affected predation in 2 out of 8 rounds and parasitism in 1 out of 8 rounds. Similarly, across the 7 crops in 2018 to 2020, exposure time significantly increased predation in 4 out of 18 rounds and parasitism in 5 out of 18 rounds. In plum, peach, tomato, and sassafras, when significant differences were detected for parasitism, egg masses deployed for 5 d resulted in higher percent parasitism compared to 2 d. In tomato, 3 d of exposure led to a higher percent parasitism than the 2 d treatment. Higher parasitism with 3 d compared to the 2 d treatment may have been due to a combination of moderate predation and smaller plant size, but this needs further evaluation. In sassafras, 4 d of exposure was similar to the 5 d treatment. In plum and soybean, when percent predation increased over the season, higher predation was detected 5 d after deployment compared to 2 d.

Except for 2 new records, *Tr. solocis* and *Tr. basalis* (Balusu et al. 2019a, b), each native parasitoid species that emerged from brown marmorated stink bug eggs in our study has been reported to parasitize eggs of this pest in other regions of the US (Cornelius et al. 2016a, b; Herlihy et al. 2016; Ogburn et al. 2016; Dieckhoff et al. 2017; Jones et al. 2017). Prevalence of parasitoid species that emerged from brown marmorated stink bug eggs was primarily habitat specific, as previously reported for brown marmorated stink bug and native stink bug species (Okuda & Yeargan 1988; Cornelius et al. 2016a; Herlihy et al. 2016; Tillman 2016; Jones et al. 2017). *Anastatus reduvii*, *Tr. brochymenae*, and *Tr. euschisti* were prevalent in woody habitats (i.e., plum, peach, and sassafras). *Ooencyrtus* sp. was the primary species emerging from brown marmorated stink bug eggs in tomato, soybean, and corn. *Gryon obesum* and *Tr. basalis* and *Te. podisi* parasitized brown marmorated stink bug eggs in these crops when egg masses of *N. viridula* and *Euschistus servus* (Say) (Hemiptera: Pentatomidae), respectively, were attacked by these parasitoids.

The type of predation damage detected for brown marmorated stink bug sentinel egg masses varied by crop. Complete and incomplete chewing, punctured sucking, and hole sucking predation were

detected for eggs deployed in plum and sassafras. As brown marmorated stink bug nymphs develop in plum and peach over the season, they cannibalize sentinel eggs resulting in an increase in prevalence of stylet sucking feeding on eggs (Tillman et al. 2020). Nymphs likely led to the increase in predation of brown marmorated stink bug egg masses over the season in plum in the current study and have the potential to increase predation in other plants. Indeed, development of brown marmorated stink bug nymphal populations and subsequent stylet-sucking predation of sentinel egg masses by nymphs has been observed in sassafras (G. Tillman, unpublished data). In contrast to plum, predation of brown marmorated stink bug egg masses in soybean and corn potentially could reach high levels, but also was consistent over the season and across treatments. Thus, parasitism remained low over time as reported in Tillman et al. (2020). A complex of predator species prey on stink bugs, including their nymphs, in corn and soybean (Tillman 2011; Tillman et al. 2015; Morrison et al. 2016; Ogburn et al. 2016). Complete chewing, removal of egg masses, and stylet sucking represented the major predator groups in these crops. In a previous report, predation was higher than parasitism in 2 row crops, cotton and soybean, and chewing predation and egg removal were the prevalent types of predation (Tillman et al. 2020).

In 2017 and 2018, we discovered *Acroclisoides sinicus* (Huang & Liao) (Hymenoptera: Pteromalidae), a hyperparasitoid of primary scelionid egg parasitoids, emerging from naturally occurring stink bug egg masses, including a parasitized brown marmorated stink bug egg mass (i.e., eggs black) in 2017 in pecan, 3 *Chinavia hilaris* (Say) (Hemiptera: Pentatomidae) egg masses collected in mimosa 7 d after detected as fresh egg masses in 2018, and 2 *N. viridula* egg masses collected in cotton 7 d after being found as fresh egg masses (Sabbatini Peverieri et al. 2019; Giovannini et al. 2021). This hyperparasitoid also has emerged from *Euschistus* sp. and *Brochymenae* sp. egg masses in Maryland, USA (Sabbatini Peverieri et al. 2019). A preliminary exposure time test using brown marmorated stink bug sentinel egg masses in sassafras trees in a woodland habitat indicated that the duration of exposure influenced the likelihood of parasitization of scelionids by *A. sinicus* (Giovannini et al. 2021). Adult *A. sinicus* did not emerge from egg masses exposed for 3 and 4 d, 1 *A. sinicus* male emerged from egg masses exposed for 5 d, and females and males emerged from egg masses exposed for 6 d. In a laboratory study, *A. sinicus* emergence was obtained when exposing 5 d and 7 d old primary parasitized egg masses for 72 h to females of *A. sinicus* (Giovannini et al. 2021). Thus, unless sentinel egg masses are deployed in the field for at least 5 d, this hyperparasitoid species, and perhaps others, will not be detected.

Many factors can impact the rate of parasitism in the field, including timing and duration of stink bug oviposition in a host plant, parasitoid host searching behavior, parasitoid habitat preferences, activity within and between nearby crop and non-crop host plants, availability of floral resources, management practices, environmental conditions, and host and parasitoid species density and diversity. The first step a foraging female takes to parasitize a stink bug egg mass is to find suitable habitat and search for a host (Bin et al. 1993; Ponzio et al. 2016). Once a female locates a host egg mass, she assesses host suitability (Laumman et al. 2009), and if suitable, she oviposits an egg into the host egg (Wilson 1961; Weber et al. 1996). During this process, oviposition by a female can be influenced by many factors such as her age and nutritional status, the age of the stink bug eggs, presence of natural enemies and competitors, and environmental conditions such as temperature and humidity (Ohno 1987; Weber et al. 1996; Takeshi & Numata 2000; Kivan & Kilic 2004; Cusumano et al. 2012; Abram et al. 2015). Unfortunately, much of the information regarding factors that lead to variation in oviposition remains unknown when sentinel egg masses are deployed. At most, we may understand the phenology of

both brown marmorated stink bugs and suitable hosts in the landscape, host age of sentinel eggs, and environmental conditions. The nutritional status of parasitoids may be unknown even if flowering plants are present within agricultural farmscapes. One possibility for enhancing parasitism rates could be to provide nectar-producing flowers in within the agricultural farmscape (Tillman & Carpenter 2014). One suggestion to determine the presence and species of parasitoid adults and their competitors and level of predation is to conduct a sentinel egg pre-test by deploying and watching a small number of egg masses over a short period of time. Another suggestion would be to deploy yellow sticky cards to sample presence or absence of parasitoids. Also, using field cages to exclude predators and release parasitoid females of known age and mating status could result in maximizing parasitism in experiments with sentinel egg masses (Herlihy et al. 2016).

A longer deployment period in the field may provide more time for females to search for and find suitable host plants and brown marmorated stink bug sentinel egg masses, as well as provide more time to parasitize an egg mass. Currently, limited information is available on specific search times of native parasitoid species in the southeastern US. The test conducted in sassafras in 2020 revealed that although some parasitoids began to parasitize egg masses on the day of deployment, parasitism was still higher at the 5 d deployment treatment compared to 2 d. Limited information is available on oviposition behavior of egg parasitoids in the laboratory. After finding an egg mass, a *Tr. basalis* or *Trissolcus utahensis* (Ashmead) (Hemiptera: Pentatomidae) female examines several eggs by antennal palpation (Wilson 1961; Weber et al. 1996). Once she selects an egg for oviposition, she deposits her egg into the host egg. Afterwards, the female leaves a host marking pheromone on the egg that deters her and other females from ovipositing into the egg (Wilson 1961; Bin et al. 1993; Colazza et al. 1996). Oviposition continues until all eggs within the mass are parasitized. The duration of the process of oviposition for *Trissolcus* spp. ranges from 2 to 15 min (Wilson 1961; Ohno 1987; Weber et al. 1996). Host marking takes only 14 to 25 s for *Tr. basalis* (Wilson 1961). The time spent by a *Tr. basalis* female to select the next host for oviposition takes up to 2 min. At the end of parasitization of the egg mass, the female can search for unmarked eggs for 15 or more min before abandoning the search. The majority of brown marmorated stink bug egg masses have 28 eggs (Nielsen & Hamilton 2009). Based on timing of oviposition in the laboratory, the duration of oviposition could range from 2.2 to 7.3 h for a brown marmorated stink bug egg mass in the field. In addition, during oviposition, competition by conspecific females can occur, and driving a female from the egg mass may occupy about 10 min (Wilson 1961). In the current study, individual egg masses rarely were parasitized completely, indicating that parasitoids were competing with other natural enemies for egg masses. In plum and sassafras, percent parasitism per egg mass was higher for the longer duration of deployment in the field, suggesting that the increase in deployment time may have provided parasitoids more time to find and parasitize individual egg masses, but this needs further investigation.

Overall, our results strongly suggest that 5 d is the optimal deployment time to assess percent predation and parasitism of brown marmorated stink bug egg masses in the field. Even though significant differences in deployment times were not detected for each round in crops, the trend over all crops showed that percent predation and parasitism was higher at 5 d deployment times compared to 2 d and 3 d exposure times. Also, the hyperparasitoid *A. sinicus* cannot be detected at deployment times lower than 5 d. Furthermore, naturally occurring egg masses are exposed to primary parasitoids and predators for approximately 5.5 to 6 d (G. Tillman, unpublished data). Perhaps exposure duration for sentinel egg masses should attempt to mimic naturally occurring egg masses, especially given the variability of factors that affect parasitism and predation under field conditions.

## Acknowledgments

This work was supported by the United States Department of Agriculture National Institute of Food and Agriculture [grant numbers 2016-51181-25409, 2018-700006-27172], and the Alabama Agricultural Experiment Station, Prattville, Alabama, USA. We thank Xuelin Luo (University of Georgia) for statistical support, and Kristie Graham, Merry Bacon, Rebekah Hartley (USDA, ARS), Katherine Cassell, Sarah Hobby, and Colby Nordstrom (University of Georgia), and Jarrett Price, Brady Keese, and Gamze Mertoglu (Auburn University) for excellent technical support. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture or any other author affiliated institution.

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