N O T E

Establishment of *Tiphia vernalis* (Hymenoptera: Tiphiidae), a Naturalized Parasitoid of the Japanese Beetle, *Popillia japonica* (Coleoptera: Scarabaeidae), in Meramec State Park, Sullivan, Missouri, USA[1]

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The Japanese beetle, *Popillia japonica* Newman, was introduced accidentally from Japan into the United States in 1916 near Trenton, NJ (Clausen et al. 1927, USDA Tech. Bull. No. 1429). Missouri has detected populations of the Japanese beetle since 1934, just 18 yr after the initial establishment in New Jersey. During the 1930s, the extent of Japanese beetle population in Missouri was limited mainly to areas in the city of St. Louis, around the Forest Park area. By 1999 (when this biocontrol project was initiated), large populations of the Japanese beetle were found at only three sites in Missouri: (a) Meramec State Park in Sullivan, (b) a nursery center in the Kansas City area, and (c) Hidden Valley Golf Course in Stone County near Boaz, MO (Brown 1996, Proc. 1996 Ann. Japanese Beetle Rev., McMinnville, TN).

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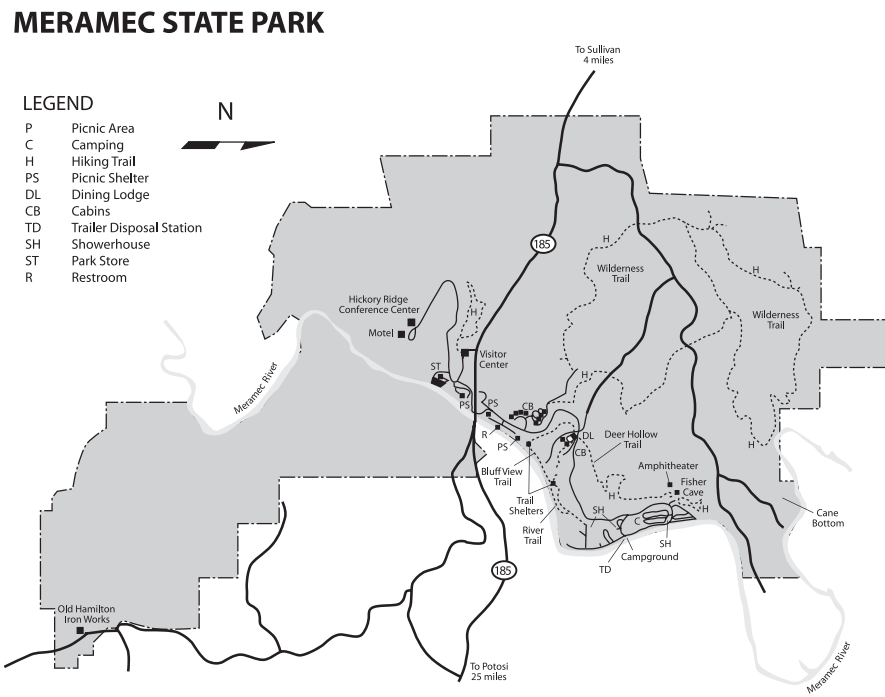

Fig. 1. Map of Meramec State Park, Missouri, showing the location of the picnic shelter areas (PS) where 103 female *Tiphia vernalis* wasps were released in April of 1999. All monitoring and subsequent recoveries of *T. vernalis* and associated tiphiid wasps were made in this picnic area on the north side of Highway 185.

In 1986, the Japanese beetle infestation in Meramec State Park, Sullivan, MO (N 38°13′19.99″, W 91°4′30″) was discovered in the picnic shelter (PS) area. This is an environmentally sensitive area near Fisher Cave and next to the Meramec River (Fig. 1). The application of broad-spectrum insecticides to control the beetle could have detrimental effects to other park fauna in this area. Therefore, least toxic and biological methods were employed by the park staff to suppress populations of the beetle (B. Wilcox, Missouri Dept. of Natural Resources, pers. comm.). By 2009, populations of the beetle were widespread, and Missouri was considered generally infested in regulatory status.

In the 1920s, USDA researchers in Japan and Korea originally identified the tiphiid wasp *Tiphia vernalis* Rohwer as a significant biological control agent of both the Japanese beetle and Oriental beetle, *Exomala* (formerly *Anomala*) *orientalis* (Waterhouse) (Gardner and Parker 1940, USDA Tech. Bull. No. 738). Between 1925 and 1927, *T. vernalis* was released in numerous areas of the northeastern United States and quickly became established as a significant natural enemy of Japanese beetle populations (Clausen et al. 1932, USDA Tech. Bull. No. 308). Reding and Klein (2001, Great Lakes Entomol. 34: 67–68) found T. vernalis wasps

parasitizing Oriental beetle grubs in Ohio nurseries. Surveys by Ramoutar and Legrand (2007, Fla. Entomol. 90: 780–782) in Connecticut during 2007 found *T. vernalis* present in every county with wasps present in areas where they were not originally released. They also found the wasps were attracted mainly to peonies. Rogers and Potter (2004, Environ. Entomol. 33: 520–527) found *T. vernalis* parasitizing Japanese beetle grubs in Kentucky at rates as high as 58%.

Tiphia vernalis was historically effective in suppressing outbreak populations of Japanese beetles (King and Parker 1950, USDA, Agric. Res. Admin. Publ. E-799). In areas with appropriate food plants (mainly umbels [Apiaceae] like wild parsnip, *Pastinaca sativa* L., and other plants like peonies, *Paeonia lactiflora* L.), the wasp parasitized an increasing percentage ($\geq$65%) of the Japanese beetle larval population, thus causing these populations to be diminished over a period of several years (Clausen 1956, USDA Tech. Bull. No. 1139). Surveys in Pennsylvania and New Jersey during 1935–1949 found parasitization of Japanese beetle larvae by *T. vernalis* ranging as high as 61% (Gardner 1938, J. Econ. Entomol. 27: 497–499). Percentage of parasitization of grubs increased as the host population density increased: 57% for 6 grubs/0.09 m^3, 31% for 2 grubs/0.09 m^3, and $<$20% for 1 grub/0.09 m^3 (King and Parker. 1950).

Planting or the occurrence of additional tiphiid food plants in the areas where beetle larvae consistently occur, e.g., golf courses, parks, and the areas surrounding airports, could increase these percentages of parasitization significantly (Clausen et al. 1933. USDA Tech. Bull. No. 366). The USDA Animal and Plant Health Inspection Service (APHIS) initiated a biologically-based integrated pest management program against Japanese beetles at regulated airports in 1996 (Tanner et al. 1997, Reg. Rev. Japanese Beetle/Pine Shoot Beetle, Louisville, KY).

In Ji'nan Province of the People's Republic of China, Luo and Jiang (1996, Chin. J. Biol. Control 12: 156–160) successfully used the knowledge of tiphiid food plants detailed in older USDA publications on Japanese beetle biocontrol to produce a video and report to show how to increase the rates of *Holotrichia* spp. scarab larvae parasitized by *Tiphia popilliavora* Rohwer and *Tiphia phyllophagae* Allen & Jaynes to an average rate of 85% in peanuts (*Arachis hypogaea* L.). We (Klein and McDonald) observed firsthand from Luo and Chiang the operational field use of tiphiid food plants to increase parasitization of white grubs in the Ji'nan, China, area in the 1990s. This provided us with validation of the practice of using food plants to increase parasitization by tiphiids of white grubs.

On 21 April 1999, prerelease surveys of Japanese beetle larval populations in Meramec State Park were conducted at potential wasp release sites having abundant mowed turf on the north side of Highway 185 (playground, central picnic area [PS], and west picnic area [PS] (Fig. 1). The prerelease surveys consisted of obtaining nine soil samples of 0.09 m^3 each, each separated by 3 m in a 3×3 "tic-tac-toe–like" pattern. In order to determine which area had the highest density of Japanese beetle larvae, soil surveys were conducted in three separate sites in the park's day-use picnic area. The area having the highest Japanese beetle larval density was selected for the release of the *T. vernalis* wasps.

In cooperation with Tennessee State University's Otis L. Floyd Nursery Research Center (McMinnville, TN), 103 female *T. vernalis* were collected from a nursery near McMinnville on 20 April 1999. Collection, storage, and release methods described by King and Parker (1950) were used to hold and transport

wasps. The adult wasps were released at Meramec State Park the next day, 21 April 1999.

The west end of the picnic area (PS) had the highest number of Japanese beetle larvae (1.4 larvae/0.09m^3) present, as determined from the previously described soil samples (Fig. 1; Table 1), and was selected for the release of the wasps. In order to provide an initial food source for the wasps and to discourage dispersal of the wasps from the area, nearby foliage was sprayed with water containing a 10% (w/v) sucrose solution just prior to release. One hundred three female *T. vernalis* wasps were released into this area on 21 April 1999.

Parasitization rates of the Japanese beetle by *T. vernalis* were calculated through soil surveys performed in a 2-wk period between the time the *T. vernalis* wasps cease egg laying and the adult Japanese beetles emerge. The surveys consisted of digging and sifting the soil in the survey area and collecting all stages of the beetle and parasitoids present. The ratio of tiphiid cocoons to Japanese beetle life stages provided a percentage of parasitization of Japanese beetle larvae by *T. vernalis* as described by King and Parker (1950). Japanese beetle larval presence was detected by soil surveys in the three previously designated areas (west picnic, cabins, and central picnic). The 1999 survey originally consisted of taking nine soil samples of 0.09 m^3 each and sifting through the soil to find Japanese beetle larvae, pupae, adults, and associated *Tiphia* cocoons. From 2000 on, the number of soil samples was increased to 20 soil samples of 0.09 m^3 each in a 4×5 sample grid pattern of 9.14×12.19 m.

In 1999, prerelease Japanese beetle larval levels in the picnic areas were low, averaging 1.4 larvae/0.09 m^3 in the west end of the picnic area, 0.11 larvae/0.09 m^3 at the cabin area near the playground, and 0.88 larvae/0.09 m^3 in the central part of the picnic area (Table 1). We first detected a single *Tiphia* cocoon in the 2000 soil survey (0.25 larvae/0.09 m^3) in the west picnic area. Three *Tiphia* cocoons were found in 2003 in the central picnic area, among a total of 25 Japanese beetle larvae. The recovery of *Tiphia* cocoons in the same area as Japanese beetle larvae and pupae indicated the possibility of *T. vernalis* establishment at a parasitization rate of 12.0% of the host density. In May 2005, we found three tiphiid cocoons and 13 larvae (18.8% parasitization rate) in the central picnic area. During the same time, we collected two *T. vernalis* females. In June 2005, we found a total of two *Tiphia* cocoons, while 12 third instars, 2 prepupae, 2 pupae, and 2 adults of Japanese beetles were found in the central picnic area, yielding a parasitization rate of $\sim$10% (Table 1).

After release of the wasps in April 1999, we field surveyed the original release site in the picnic area (PS) of Meramec State Park for tiphiid wasp presence in five separate years as funds were available for monitoring: 2000, 2003, 2005, 2017, and 2018 (Fig. 1). A 10% (w/v) sucrose solution was sprayed on broadleaf foliage during sunny calm days to attract wasps, which requires several hours of weather with a light wind to disperse the scent of sugar. Wasps were attracted to the sugar solution and were captured for identification and preliminary oviposition behavior observations on Japanese beetle larvae to identify the wasp to species level. An oviposition site in the suture between the third thoracic segment (T3) and first abdominal (A1) is characteristic of this species (Clausen et al. 1933).

We were unable to find any *Tiphia* spp. adults during the field survey of May 2000. On 18 May 2005, 8 female *Tiphia* wasps and 10 male wasps were captured

Table 1. Soil survey life stage counts at Meramec State Park picnic area of the Japanese beetle and associated tiphiid cocoons.

Meramec State Park Picnic Area	Date	No. of 0.09-m^3 Soil Samples	Third Instars	Pupae	Adult Beetles	*Tiphia* Cocoons	Life Stages/ 0.09 m^3	Percentage Parasitization*
Playground	21 April 1999	9	1	0	0	0	0.11	0.0
West	21 April 1999	9	13	0	0	0	1.4	0.0
West	27 May 1999	9	5	0	0	0	0.55	0.0
West	20 June 2000	20	1	1	2	1	0.25	20.0
Central	21 April 1999	9	8	0	0	0	0.88	0.0
Central	27 May 1999	9	5	0	0	0	0.56	0.0
Central	20 June 2000	20	0	0	0	0	0.0	0.0
Central	20 June 2003	20	1	17	4	3	1.25	12.0
Central	18 May 2005	20	13	0	0	3	0.8	18.8
Central	2 June 2005	20	14	2	2	2	1.0	10.0

* Percentage parasitization calculated as (sum of *Tiphia* cocoons/sum of Japanese beetle life stages).

Table 2. Field vegetation survey results for *Tiphia vernalis* wasps in Meramec State Park, Sullivan, MO. A 10% table sugar water attractant was sprayed on broad-leaved plants, and a minimum of 4 h of field collections were performed in the picnic area on the north side of Highway 185. *Tiphia vernalis* was first found to be established in 2005, 6 yr after its release. Both years sampled thereafter (2017 and 2018) were positive for *T. vernalis*. All *T. vernalis* wasps captured were females.

Field Survey Date	Total *Tiphia* Adults	*Tiphia vernalis*	*Tiphia infossata*	*Tiphia letalis*
9 May 2000	0	0	0	0
20 May 2003	2	0	2	0
18 May 2005	18	2	15	1
26 May 2017	3	1	1	1
24 May 2018	3	3	0	0
25 May 2018	3	3	0	0
Total	29	9	18	2

(Table 2). Two of the female wasps readily oviposited on third instars in the characteristic egg location for *T. vernalis*. Taxonomic identifications confirmed the identity of the two female wasps as *T. vernalis*; the other six females were *Tiphia infossata* Allen, and all males were *T. infossata*, except for one *Tiphia letalis* Roberts (Table 2). Positive recovery of *T. vernalis* from the 1999 release was made 6 yr after release of 103 adult females, inferring establishment of the wasp. *Tiphia letalis* is a parasitoid of native ground beetle larvae; the host of *T. infossata* is unknown, but presumed to be a beetle larva.

Beginning in 2017, we reactivated the Japanese beetle biological control program with the Oregon Department of Agriculture using *T. vernalis*. Adult *T. vernalis* surveys were performed at Meramec State Park in May of both 2017 and 2018 in order to determine the status of the *T. vernalis* wasp there. On the 26 May 2017, three *Tiphia* wasps were captured, including two females and one male (Table 2). One of the female wasps readily oviposited on a third-instar Japanese beetle in the characteristic location for *T. vernalis* (in the T3–A1 suture). Taxonomic identifications confirmed the identity of this female wasp as *T. vernalis*; the other female was *T. infossata*, and the one male *Tiphia* was identified as *T. letalis*. The collection of the female *T. vernalis* confirms positive establishment of this species 18 yr after the 1999 release. In late May 2018, we captured six more female *T. vernalis* in 2 d of collecting, as evidenced by oviposition of an egg in the characteristic location between the T3–A1 suture of Japanese beetle larva during overnight sting setups of the collected wasps in 240-ml soil tins.

Our survey efforts indicate *T. vernalis* is established in low numbers at Meramec State Park, Sullivan, MO. We have had positive field recoveries of adult female *T.*

vernalis wasps in Meramec State Park in 2005, 2017, and 2018 from a single release of 103 female wasps in 1999. We also recently received confirmation in August 2019 of the establishment of both the spring tiphia and winsome fly, *Istocheta aldrichi* (Mesmil) (Diptera: Tachinidae) in the Minneapolis–St. Paul area from this same biocontrol program performed with APHIS against Japanese beetle populations in 2003 in Minnesota (J. Hahn, Minnesota Extension Service, pers. comm.).

The establishment of spring tiphia wasps in Missouri and Minnesota from releases made in 1999 and 2003, respectively, are the first known establishments from intentional releases of Japanese beetle parasitoids on the western side of the Mississippi River. Establishment of the spring tiphia wasp in Minnesota and Missouri validates our recapitulation and implementation of the older published operational field knowledge concerning the spring tiphia.

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