

DETERMINING THE ESTABLISHMENT POTENTIAL
OF *GANASPIS KIMORUM* IN MICHIGAN

By

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ABSTRACT

Drosophila suzukii is an invasive pest that has disrupted existing IPM strategies of cherry and small fruit industries. Shortly after its introduction, *D. suzukii* established across the United States, by exploiting non-crop habitats and infesting thin-skinned fruit monocultures. Using its serrated ovipositor, females can infest wild and cultivated fruit throughout the growing season. In Michigan, native predators are insufficient for suppressing *D. suzukii* populations, creating a dependency on chemical sprays. However, populations residing in wild refugia adjacent to agricultural fields are left untreated and require alternative methods. *Drosophila suzukii* natural enemies within its native range were investigated, in search for a classical biocontrol agent. *Ganaspis kimorum* (formerly *G. brasiliensis*) was found to be an exclusive larval parasitoid of *D. suzukii* and a potential self-regulating method of targeting pest populations. I investigated the ability of *G. kimorum* to overwinter in Michigan fields, near locations of release and the abiotic factors that influence its success. Snow cover and number of days where wasps were exposed to ground temperatures below freezing influenced survival. I also evaluated *G. kimorum* dispersal capability and host finding behavior using baited traps just after harvest; however, no *G. kimorum* was detected in sentinel traps. Specimens of this species were found in fruit surveys conducted at release sites, along with adventive populations of *Leptopilina japonica*. This is the first detection of *G. kimorum* emerging from established *D. suzukii* in Michigan agricultural fields. These findings will be influential in determining the future success of establishing *G. kimorum* as a biocontrol agent against *D. suzukii* populations.

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Chapter 1: Towards the sustainable management of *Drosophila suzukii*

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is an invasive pest that has had detrimental effects on cherry and small fruit industries by disrupting existing integrated pest management (IPM) programs and driving pest management decisions close to harvest. Since its first detection in 2010, *D. suzukii* has established in Michigan by utilizing non-crop habitats to develop and then to infiltrate thin-skinned fruit monocultures close to harvest. Unfortunately, native natural enemies do not provide enough population suppression to regulate communities. Hence, the arrival of this pest has caused an increase in chemical pesticide use in susceptible cropping systems impacted. Recently, classical biocontrol has been a focus of study, as a novel parasitoid from *D. suzukii*'s native range has been permitted for rearing and release. The larval parasitoid, *Ganaspis kimorum* Buffington (previously *G. brasiliensis*), is a potential biocontrol agent to be released for establishment in Michigan to regulate *D. suzukii* populations.

Invasive species and their impact

Increased instances of invasive species are a byproduct of globalization and trade (Hulme et al., 2008, Seebens et al., 2017). The movement of goods has allowed for multiple introductions of exotic species into a new environment and establish into a new region (Hulme et al., 2009). Exotic species that become invasive arrive with evolutionary traits from their native range that allow them to occupy empty niches or outcompete native organisms (Williamson & Fitter, 1996). Faster generation cycles, functional trait plasticity, reduced predation, and a higher number of introduction pathways leverage an advantage against native species (Roy et al., 2011, Zhao et al., 2023). Without community regulation these organisms have the potential to cause harm to the environment and/or economy (Pimental et al., 2001, Kenis et al., 2009).

***Drosophila suzukii*: Native range and biology**

Drosophila suzukii is native to Southeast Asia where it thrives in moderate temperate climates with high humidity (Kanzawa, 1939, Tochen et al., 2016). Commonly named after the male's prominent marking on its wing, spotted-wing drosophila (SWD), is a generalist on many soft and thin-skinned fruits (Kikkawa and Peng 1938, Walsh et al., 2011, Lee et al., 2015).

Drosophilids native to North America feed on sugars from decaying material, but *D. suzukii* females use a serrated ovipositor to slice into ripening fruit (Lee et al., 2011). This allows for the infestation of fruit prior to harvest, including cultivated crops such as blueberry, tart cherry, raspberry, and grapes (Lee et al., 2015). Its host range also includes wild berry producing plants present in natural areas, like pokeweed, elderberry, and blackcaps (Lee et al., 2015, Klick et al., 2016, Leach, et al., 2019).

Once fruit has been infested, the egg will take roughly 48 hours to develop into a 1st instar larva. Then the larva will feed within the fruit, advancing through 3 instars before pupating over the course of approximately 6 days depending on ambient temperatures (Emiljanowicz et al., 2014). Puparia drop to the ground, often while still within host fruit, where they will continue developing for about a week (Walsh et al., 2011). *Drosophila suzukii* undergo complete metamorphosis, emerging as adults that can live for approximately one month, with an egg laying potential of up to 600 eggs per female (Emiljanowicz et al., 2014, Reynolds et al., 2019). *Drosophila suzukii* populations start building in late spring, producing up to 13 generations per year in Japan and Oregon (Kanzawa 1939, Tochen et al., 2014).

Detection and management practices

Upon its arrival in the United States in 2008, *D. suzukii* spread from California to the east coast within three years and is now established across the U.S. (Asplen et al., 2015). The species'

success can be attributed to its rapid reproduction, serrated ovipositor, lack of natural enemies, and use of a variety of substrates (Lee et al., 2011, Haye et al., 2016). In 2010, *D. suzukii* was estimated to cause over \$500 million in damage to berry and cherry industries on U.S. West Coast and caused an increase in insecticide use by 80% in some berry cropping systems in 2014 (Bolda et al., 2010, Walsh et al., 2011, Burrack, 2013, Van Steenwyk et al., 2014).

Current management relies on pesticide sprays that target adults (Beers et al., 2011, Bruck et al., 2011). Challenges arise due to most processors having a zero tolerance for larval detection in processed fruit, and larvae residing within fruit being protected from insecticidal sprays (Van Timmeren and Isaacs, 2013, Shower et al., 2018). In addition, the majority of sprays are broad-spectrum insecticides that harm natural enemies of *D. suzukii*, reducing potential predators and parasitoids. Increased exposure to a range of sprays has increased *D. suzukii* tolerance towards chemical control methods and increases their ability to become resistant to others (Beers et al., 2016, Van Timmeren et al., 2019). Therefore, alternative methods of control are needed to increase sustainability while regulating populations.

Cultural management strategies that can reduce or prevent fruit infestation by *D. suzukii* have been studied (as reviewed by Tait et al., 2021). Pruning the canopies of host plants to increase air flow (Haye et al., 2016) or using drip irrigation instead of overhead sprinklers (Rendon et al., 2019) have been found to reduce humidity in the crop canopy, making it less favorable to *D. suzukii*. Physical barriers such as applying exclusion netting over crop canopies to reduce fruit infestation (Cormier et al., 2015, Leach et al., 2016) or placing mulch beneath crop canopies to make the ground inhospitable for pupae to develop into adults (Schöneberg et al., 2020), show promise in systems where they are practical to implement. However, these practices are not universally feasible across all susceptible crops or farm scales.

Since its invasion, the behavior and physiology of *D. suzukii* has been well studied to understand its seasonable phenology. Population modeling and trapping has allowed for anticipating growth patterns and efficiently applying interventions at critical time points (Drummond et al., 2019, Wilson et al., 2022). Attractant and repellent olfactory cues have been used to draw *D. suzukii* away from crops, either by attracting flies to insecticide treated substrates, which reduces lethality to non-target species, or by repelling *D. suzukii* away from susceptible crops (Klick et al., 2019, Tait et al., 2021). Another area of active research involves manipulating *D. suzukii* at the molecular level as a control strategy, by producing sterile males to compete with wild forms or introducing RNA interference genes through baits (Tait et al., 2021). Though these strategies have been successful with other pest groups, the ethical use and potential risks associated are still under review (Tait et al. 2021).

Long term solutions for reducing *D. suzukii* populations include the identification, introduction, and establishment of biological control agents, a sustainable strategy that benefits the environment and has the potential to decrease the financial burden on growers who must prevent fruit infestation. Organisms that are known to suppress *D. suzukii* include predators, bacteria, fungi, nematodes, and parasitoids (Tait et al. 2021). While their contributions alone may not provide the necessary level of prevention, it is thought that effective control could be achieved when implemented together (Abram et al., 2020, Tait et al., 2021). Parasitoids have the potential to be self-sustaining since they respond to the population growth of their host, are energy efficient in terms of host seeking, and resilient to the immune response in co-evolved hosts (Vinson, S.B.1976, Vet & Dicke, 1992). Native parasitoids struggle to overcome the immune system of *D. suzukii*, but exotic parasitoids from its native range have greater chances for survival (Fleury et al., 2009, Kacsoh & Schlenke, 2012, Lee et al., 2019).

Potential agents for biological control

In the search for more sustainable management options, surveys were conducted in the native range of *D. suzukii* to collect candidate parasitoids for study. Parasitoids were reviewed by the USDA for host specificity and to ensure minimal interaction with native biota. Of these, several were shown to be generalists of drosophila species and were eliminated for high risk (Daane et al., 2016, Girod et al., 2018, Giorgini et al., 2019). Additional studies were conducted on three parasitoid wasps, *Asobara japonica* (Belokobylskij) (Hymenoptera: Braconidae), *Leptopillina japonica* (Novkovic & Kimura) (Hymenoptera: Figitidae), and *Ganaspis brasiliensis* (Ihering) (Hymenoptera: Figitidae) (Girod et al., 2018, Wang et al., 2019). Further studies showed populations of *A. japonica* parasitized up to 5 non-target hosts, while populations of *L. japonica* successfully parasitized up to 3 populations of drosophila (Girod et al., 2018). For *G. brasiliensis*, populations collected from different locations showed a strong variation in host specificity and were hence separated into five groups by locality of origin (G1-G5), of which G1 showed to be the most host specific, exclusively targeting *D. suzukii* (Girod et al., 2018, Nomano et al., 2017). The G1 strain of *G. brasiliensis* was subsequently approved for rearing and release out of quarantine by the USDA Animal and Plant Health Inspection Service (APHIS) in 2021. Michigan Department of Agriculture and Rural Development (MDARD) approved its rearing and release in 2022 for the purposes of establishing populations of the parasitoid in susceptible fruit production areas. In May 2024 *Ganaspis brasiliensis* was renamed as *Ganaspis kimorum* Buffington (Hymenoptera: Figitidae) to reflect its population (G1) being identified as a separate species (Sosa-Calvo et al., 2024).

***Ganaspis kimorum* biology and adventive populations**

Ganaspis kimorum is a parasitoid of *D. suzukii*, native to Japan. The wasp belongs to the Figitidae family, a diverse group of parasitoid wasps ranging from over 1,500 different species. The wasp targets larvae in ripe fruit, using their ovipositor to parasitize soft body L2 larvae (~35h) (Emiljanowicz et al., 2014, Wang et al., 2018). The parasitoid wasp larvae takes advantage of *D. suzukii*'s development by remaining dormant for 96h in the egg stage, before emerging and feeding within *D. suzukii* larvae for 24-48hrs. Encased in the puparia and continuing to feed as an endo-parasite (Wang et al., 2018), *Drosophila suzukii* pupate 120-144 h later (Emiljanowicz et al., 2014). Successful wasps will continue to develop within the puparia for 3-4 weeks before emerging as adults. Parasitoid wasps are known to use pupariums of their hosts to survive natural conditions while developing (Reynolds et al., 2019). Male *G. kimorum* wasps tend to emerge 5-7 days before females, giving them an opportunity to mature and feed on nearby nectar resources and sexually mature. Female wasps emerge later and are sexually mature within 3 days, depositing roughly 80 eggs over the course of their lifespan. Females live for 3-4 weeks as adults, while males tend to expire after 2 weeks (Wang et al., 2018).

While quarantine studies were still underway, adventive populations of *G. kimorum* were found in the southwest region of British Columbia, Canada in 2020 (Abram et al., 2020). The following year, the wasp was reared from infested blackberry in Washington, United States (Beers et al., 2022). Unfortunately, these adventive populations are not permitted to be used in rearing and release programs.

Establishment efforts in the U.S.

As a coordinated effort across 14 states, *G. kimorum* was reared in colony to be released for establishing it where susceptible crops are grown. In 2024, nearly 140,000 wasps were

released in the U.S. in a variety of systems. In Michigan nearly 40,000 wasps were released at 30 sites near commercial, research, or unmanaged tart cherry or blueberry plantings (Wilson and Isaacs, unpublished). Releases were done in wooded edges adjacent to susceptible host crops with a history of *D. suzukii* pressure close to harvest. Natural areas were surveyed prior to releases to detect hosts of *D. suzukii* and to better understand potential establishment opportunities for *G. kimorum* outside of release zones. Further surveys of parasitoid communities will continue to determine *G. kimorum* presence as well as monitor *D. suzukii* communities to associate fluctuations with the integration of natural enemies. Similar to *D. suzukii*, the expectation is that *G. kimorum* will become established across the continental U.S. where climate and topography are similar to its native range of southeast Asia (Wan & Yang, 2015).

Thesis objectives

The goal of this thesis was to study factors relevant to the establishment of *G. kimorum*, currently being reared and released in Michigan as a classical biological control agent against *D. suzukii*. The second chapter explores its dispersal potential at Michigan blueberry and tart cherry farms. The third chapter is an exploration of abiotic conditions related to overwintering survival.

LITERATURE CITED

- Abram, P. K., McPherson, A. E., Kula, R., Hueppelsheuser, T., Thiessen, J., Perlman, S. J., Curtis, C. I., Fraser, J. L., Tam, J., Carrillo, J., Gates, M., Scheffer, S., Lewis, M., & Buffington, M. (2020). New records of *Leptopilina*, *Ganaspis*, and *Asobara* species associated with *Drosophila suzukii* in North America, including detections of *L. japonica* and *G. brasiliensis*. *Journal of Hymenoptera Research*, 78, 1–17.
- Asplen, M. K., Anfora, G., Biondi, A., Choi, D. S., Chu, D., Daane, K. M., Gilbert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Véték, G., Vogt, H., Walton, V.M., Yu, Y., Zappalá, L., & Desneux, N. (2015). Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*, 88, 469-494.
- Beers, E. H., Van Steenwyk, R. A., Shearer, P. W., Coates, W. W., & Grant, J. A. (2011). Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. *Pest management science*, 67(11), 1386-1395.
- Beers, E. H., Mills, N. J., Shearer, P. W., Horton, D. R., Milickzy, E. R., Amarasekare, K. G., & Gontijo, L. M. (2016). Nontarget effects of orchard pesticides on natural enemies: lessons from the field and laboratory. *Biological Control*, 102, 44-52.
- Beers, E. H., Beal, D., Smytheman, P., Abram, P. K., Schmidt-Jeffris, R., Moretti, E., Daane, K. M., Looney, C., Lue, C., & Buffington, M. (2022). First records of adventive populations of the parasitoids *Ganaspis brasiliensis* and *Leptopilina japonica* in the United States. *Journal of Hymenoptera Research*, 91, 11–25.
- Bolda, M. P., Goodhue, R. E., & Zalom, F. G. (2010). Spotted wing drosophila: potential economic impact of a newly established pest. *Agricultural and Resource Economics Update*, 13(3), 5-8.
- Bruck, D. J., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., DeFrancesco, J., Gerdeman, B., & Spitler, H. (2011). Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. *Pest Management Science*, 67(11), 1375-1385.
- Burrack, H. (n.d.). *Spotted-Wing Drosophila Impacts, 2013*. NC State Extension.
- Cormier, D., Veilleux, J., & Firlej, A. (2015). Exclusion net to control spotted wing *Drosophila* in blueberry fields. *IOBC-WPRS Bull*, 109, 181-184.
- Daane, K. M., Wang, X. G., Biondi, A., Miller, B., Miller, J. C., Riedl, H., Shearer, P.W., Guerrieri, E., Giorgini, M., Buffington, M., Achterberg, K.V., Song, Y., Kang, T., Yi, H., Jung, C., Lee, D.W., Chung, B.K., Hoelmer, K.A., & Walton, V. M. (2016). First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents. *Journal of Pest Science*, 89, 823-835.

- Drummond, F., Ballman, E., & Collins, J. (2019). Population dynamics of spotted wing *Drosophila* (*Drosophila suzukii* (Matsumura)) in Maine wild blueberry (*Vaccinium angustifolium* Aiton). *Insects*, 10(7), 205.
- Emiljanowicz, L. M., Ryan, G. D., Langille, A., & Newman, J. (2014). Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet. *Journal of Economic Entomology*, 107(4), 1392–1398.
- Fleury, F., Gibert, P., Ris, N., & Allemand, R. (2009). Chapter 1 Ecology and life history evolution of frugivorous drosophila parasitoids. *Advances in Parasitology*, 3–44.
- Giorgini, M., Wang, X. G., Wang, Y., Chen, F. S., Hougardy, E., Zhang, H. M., Chen, Z.Q., Chen, H.Y., Liu, C.X., Cascone, P., Formisano, G., Carvalho, G.A., Biondi, A., Buffington, M., Daane, K.M., Hoelmer, K.A., & Guerrieri, E. (2019). Exploration for native parasitoids of *Drosophila suzukii* in China reveals a diversity of parasitoid species and narrow host range of the dominant parasitoid. *Journal of Pest Science*, 92, 509-522.
- Girod, P., Lierhmann, O., Urvois, T., Turlings, T. C., Kenis, M., & Haye, T. (2018). Host specificity of Asian parasitoids for potential classical biological control of *Drosophila suzukii*. *Journal of Pest Science*, 91, 1241-1250.
- Haye, T., Girod, P., Cuthbertson, A. G. S., Wang, X. G., Daane, K. M., Hoelmer, K. A., Baroffio, C., Zhang, J.P., & Desneux, N. (2016). Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. *Journal of Pest Science*, 89, 643-651.
- Hulme, P. E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, P., Pyšek, P., Roques, A., Sol, D., Solarz, W., & Vilà, M. (2008). Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *Journal of Applied Ecology*, 45(2), 403-414.
- Hulme, P. E. (2009). Trade, transport, and trouble: Managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, 46(1), 10–18.
- Kacsoh, B. Z., & Schlenke, T. A. (2012). High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. *PloS One*, 7(4), e34721.
- Kanzawa, T. (1939). Studies on *Drosophila suzukii* Mats. Kofu, Yamanashi Agricultural Experiment Station, 49 pp. *Review of Applied Entomology*, 29, 622 (abst.).
- Kenis, M., Auger-Rozenberg, M. A., Roques, A., Timms, L., Péré, C., Cock, M. J., Settele, J., Augustin, S., & Lopez-Vaamonde, C. (2009). Ecological effects of invasive alien insects. *Biological Invasions*, 11, 21-45.

- Kikkawa, H., & Peng, F. T. (1938). *Drosophila* species of Japan and adjacent localities. *Japanese Journal of Zoology*, 7, 507-552.
- Kimura, M. T. (2004). Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia*, 140, 442-449.
- Klick, J., Yang, W. Q., Walton, V. M., Dalton, D. T., Hagler, J. R., Dreves, A. J., Lee, J.C., & Bruck, D. J. (2016). Distribution and activity of *Drosophila suzukii* in cultivated raspberry and surrounding vegetation. *Journal of Applied Entomology*, 140(1-2), 37-46.
- Klick, J., Rodriguez-Saona, C. R., Cumplido, J. H., Holdcraft, R. J., Urrutia, W. H., Da Silva, R. O., Borges, R., Mafra-Neto, A., & Seagraves, M. P. (2019). Testing a novel attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae) management. *Journal of Insect Science*, 19(1), 3.
- Leach, H., Van Timmeren, S., & Isaacs, R. (2016). Exclusion netting delays and reduces *Drosophila suzukii* (Diptera: Drosophilidae) infestation in raspberries. *Journal of Economic Entomology*, 109(5), 2151-2158.
- Leach, H., Hagler, J. R., Machtley, S. A., & Isaacs, R. (2019). Spotted wing drosophila (*Drosophila suzukii*) utilization and dispersal from the wild host Asian bush honeysuckle (*Lonicera* spp.). *Agricultural and Forest Entomology*, 21(2), 149-158.
- Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Van Steenwyk, R. A., & Yorgey, B. M. (2011). The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Management Science*, 67(11), 1358–1367.
- Lee, J. C., Dreves, A. J., Cave, A. M., Kawai, S., Isaacs, R., Miller, J. C., Van Timmeren, S., & Bruck, D. J. (2015). Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). *Annals of the Entomological Society of America*, 108(2), 117–129.
- Lee, J. C., Wang, X., Daane, K. M., Hoelmer, K. A., Isaacs, R., Sial, A. A., & Walton, V. M. (2019). Biological control of spotted-wing drosophila (Diptera: Drosophilidae) current and pending tactics. *Journal of Integrated Pest Management*, 10(1).
- Nomano, F. Y., Kasuya, N., Matsuura, A., Suwito, A., Mitsui, H., Buffington, M. L., & Kimura, M. T. (2017). Genetic differentiation of *Ganaspis brasiliensis* (Hymenoptera: figitidae) from East and Southeast Asia. *Applied Entomology and Zoology*, 52, 429-437.
- Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'connell, C., Wong, E., Russel, L., Zern, J., Aquino, T., & Tsomondo, T. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, 84(1), 1-20.

- Rendon, D., & Walton, V. M. (2019). Drip and overhead sprinkler irrigation in blueberry as cultural control for *Drosophila suzukii* (Diptera: Drosophilidae) in Northwestern United States. *Journal of Economic Entomology*, 112(2), 745-752.
- Reynolds, S. (2019). Cooking up the perfect insect: Aristotle's transformational idea about the complete metamorphosis of insects. *Philosophical Transactions of the Royal Society B*, 374(1783), 20190074.
- Roy, H. E., Lawson Handley, L. J., Schönrogge, K., Poland, R. L., & Purse, B. V. (2011). Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids?. *BioControl*, 56, 451-468.
- Schöneberg, T., Arsenault-Benoit, A., Taylor, C. M., Butler, B. R., Dalton, D. T., Walton, V. M., Petran, A., Rogers, M.A., Diepenbrock, L.M., Burrack, H.J., Leach, H., Van Timmeren, S., Fanning, P.D., Isaacs, R., Gress, B.E., Bolda, M.P., Zalom, F.G., Roubos, C.R., Evans, R.K., Sial, A.A., & Hamby, K. A. (2020). Pruning of small fruit crops can affect habitat suitability for *Drosophila suzukii*. *Agriculture, Ecosystems & Environment*, 294, 106860.
- Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., Kartesz, J., Kenis, M., Kreft, H., Kühn, I., Lenzner, B., Liebhold, A., Mosena, A., Moser, D., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., van Kleunen, M., Walker, K., Weigelt, P., Yamanaka, T., Essl, F. No saturation in the accumulation of alien species worldwide. *Nature Communications*, 8(1), 14435.
- Shawer, R., Tonina, L., Tirello, P., Duso, C., & Mori, N. (2018). Laboratory and field trials to identify effective chemical control strategies for integrated management of *Drosophila suzukii* in European cherry orchards. *Crop Protection*, 103, 73-80.
- Sosa-Calvo J., M. Forshage, M.L. Buffington (2024) Circumscription of the *Ganaspis brasiliensis* (Ihering, 1905) species complex (Hymenoptera, Figitidae), and the description of two new species parasitizing the spotted wing drosophila, *Drosophila suzukii* Matsumura, 1931 (Diptera, Drosophilidae). *Journal of Hymenoptera Research* 97: 441-470.
- Tait, G., Mermer, S., Stockton, D., Lee, J., Avosani, S., Abrieux, A., Anfora, G., Beers, E., Biondi, A., Burrack, H., Cha, D., Chiu, J. C., Choi, M., Cloonan, K., Crava, C. M., Daane, K. M., Dalton, D. T., Diepenbrock, L., Fanning, P., Ganjisaffar, F., Gómez, M.I., Gut, L., Grassi, A., Hamby, K., Hoelmer, K.A., Ioriatti, C., Isaacs, R., Klick, J., Kraft, L., Loeb, G., Rossi-Stacconi, M.V., Nieri, R., Pfab, F., Puppato, S., Rendon, D., Renkema, J., Rodriguez-Saona, C., Rogers, M., Sassú, F., Schöneberg, T., Scott, M.J., Seagraves, M., Sial, A., Van Timmeren, S., Wallingford, A., Wang, X., Yeh, D.A., Zalom, F.G., & Walton, V. M. (2021). *Drosophila suzukii* (Diptera: Drosophilidae): A decade of research

- towards a sustainable integrated pest management program. *Journal of Economic Entomology*, 114(5), 1950–1974.
- Tochen, S., Dalton, D. T., Wiman, N., Hamm, C., Shearer, P. W., & Walton, V. M. (2014). Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. *Environmental Entomology*, 43(2), 501–510.
- Tochen, S., Woltz, J. M., Dalton, D. T., Lee, J. C., Wiman, N. G., & Walton, V. M. (2016). Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry. *Journal of Applied Entomology*, 140(1-2), 47-57.
- USDA-APHIS (2021). Field Release of *Ganaspis brasiliensis* (Hymenoptera: Figitidae) for biological control of spotted-wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), in the contiguous United States. Animal and Plant Health Inspection Service of the United States Department of Agriculture.
- Van Steenwyk, R. A., & Bolda, M. P. (2014). Spotted wing drosophila: devastating effects on cherry and berry pest management. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1105* (pp. 11-18).
- Van Timmeren, S., & Isaacs, R. (2013). Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. *Crop Protection*, 54, 126-133.
- Van Timmeren, S., Sial, A. A., Lanka, S. K., Spaulding, N. R., & Isaacs, R. (2019). Development of a rapid assessment method for detecting insecticide resistance in spotted wing Drosophila (*Drosophila suzukii* Matsumura). *Pest Management Science*, 75(7), 1782-1793.
- Vet, L. E., & Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, 37(1), 141-172.
- Vinson, S. B. (1976). Host selection by insect parasitoids. *Annual Review of Entomology*, 21(1), 109-133.
- Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V. M., O’Neal, S. D., & Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, 2(1), G1–G7.
- Wan, F. H., & Yang, N. W. (2016). Invasion and management of agricultural alien insects in China. *Annual Review of Entomology*, 61(1), 77-98.

- Wang, X. G., Nance, A. H., Jones, J. M., Hoelmer, K. A., & Daane, K. M. (2018). Aspects of the biology and reproductive strategy of two Asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii*. *Biological Control*, 121, 58-65.
- Wang, X., Hogg, B. N., Hougardy, E., Nance, A. H., & Daane, K. M. (2019). Potential competitive outcomes among three solitary larval endoparasitoids as candidate agents for classical biological control of *Drosophila suzukii*. *Biological Control*, 130, 18-26.
- Williamson, M., & Fitter, A. (1996). The varying success of invaders. *Ecology*, 77(6), 1661-1666.
- Wilson, J. K., Gut, L. J., Powers, K., Huang, J., & Rothwell, N. (2022). Predicting the risk of tart cherry (*Prunus cerasus*) infestation by *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, 115(4), 1024-1028.
- Zhao, Z., Hui, C., Peng, S., Yi, S., Li, Z., Reddy, G. V., & van Kleunen, M. (2023). The world's 100 worst invasive alien insect species differ in their characteristics from related non-invasive species. *Journal of Applied Ecology*, 60(9), 1929-1938.

Chapter 2: Release, monitoring, and detection of larval parasitoids of *Drosophila suzukii*

Abstract

Ganaspis kimorum is an exotic parasitoid wasp approved for release in the United States as of 2022 that selectively targets the invasive spotted-wing drosophila, *Drosophila suzukii*. Current strategies to control *D. suzukii* rely on insecticide sprays on susceptible crops, but these are unable to suppress populations residing in adjacent non-crop habitats where the pest infests wild fruit. To target populations within wild areas bordering cultivated fruit plantings, releases of *G. kimorum* were conducted to establish a natural enemy of *D. suzukii* in Michigan. We performed a field study measuring the targeting ability of *G. kimorum* in tart cherry orchards and blueberry fields by setting baited sentinel traps in transects and collecting fruit to measure baseline parasitoid activity and the parasitoid's dispersal capacity after release. We recorded no *G. kimorum* in sentinel traps, however it was detected in fruit samples at 5 of the 16 release sites, providing the first evidence of reproduction on *D. suzukii* in Michigan. *Leptopilina japonica*, another larval parasitoid that attacks *D. suzukii* but that arrived on its own in Michigan, was also detected at 11 of the 16 sites in fruit samples or sentinel traps, indicating that this adventive species is becoming established where *D. suzukii* is abundant. *Leptopilina japonica* was most abundant at blueberry sites under organic pest management. Most of the parasitoids that were recovered emerged from *D. suzukii* infested fruit samples from the field, indicating that both species are capable of finding and parasitizing their host in a field setting.

Introduction

The invasive pest species *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) is a generalist of soft, thin-skinned fruit (Kanzawa, 1939, Lee et al., 2011). Originating from Southeast Asia, it was first detected in the United States in 2008 in California and moving across the continental states within 3 years (Asplen et al., 2015). Females possess a characteristic serrated ovipositor, which allows them to insert eggs into intact fruit (Mitsui et al., 2022, Walsh et al., 2011, Atallah et al., 2014). Now considered a key pest due to their damage and infestation of fruit close to harvest, short generation cycle, fecundity, and range of hosts, growers of susceptible fruit have become reliant on insecticides to prevent infestation by *D. suzukii* (Lee et al., 2011, Haye et al., 2016). This has created the need to reevaluate existing integrated pest management (IPM) programs (Tait et al., 2021).

Reliance on broad-spectrum insecticides within cultivated crops to offset economic losses due to this pest has increased management costs and has produced populations of *D. suzukii* resistant to common insecticides (Gress & Zalom, 2019). Alternative interventions include cultural methods (e.g., increased pruning), release of sterile males, implementing physical barriers, and use of behavior modifying compounds (e.g., attract and kill) (recently reviewed by Tait et al., 2021). Generalist predators have been studied to determine their ability to limit *D. suzukii* populations but have been found to be inadequate (Woltz et al., 2018, Lee et al., 2019, Abram et al., 2020). Current strategies target the pest once it is in the crop (Tonina et al., 2018, Wang et al., 2019a). However, the wide host range of *D. suzukii* allows it to infest wild berries in tree lines and wooded edges where it builds up populations in late spring before spilling over into adjacent crops prior to harvest (Kanzawa 1939, Tochen et al., 2014). Parasitoids offer a unique advantage in targeting *Drosophila* hosts like *D. suzukii* and may prove to be particularly

effective in attacking them in these adjacent non-crop habitats to reduce populations in the off-crop locations.

Surveys of native parasitoids that use drosophilids as hosts were conducted to evaluate parasitism rates on *D. suzukii* and determine their viability as control agents (Kacsoh & Schlenke, 2012; Lee et al., 2019). Unfortunately, there were only low levels of parasitism recorded in pupal parasitoids and little to no parasitism in larval parasitoids (Lee et al., 2019; Wang et al., 2019b). Surveys were expanded to Southeast Asia, where multiple species of parasitoid wasps were collected and studied under observation to determine their parasitism rate in *D. suzukii* and their host specificity (Daane et al., 2016, Girod et al., 2018, Nomano et al., 2017, Giorgini et al., 2019, Wang et al., 2019b). Of the species surveyed, only *Ganaspis kimorum* Buffington (formerly *G. brasiliensis* G1 strain) (Hymenoptera: Figitidae) (Sosa-Calvo et al. 2024) was determined to be sufficiently specialized as a parasitoid of *D. suzukii* and was approved for rearing and release in the contiguous United States in 2022 by USDA-APHIS.

Ganaspis kimorum seeks *D. suzukii* larvae (L2) in fruit to deposit its eggs where they hatch and feed on their host, completely consuming it after the fly larva has pupated (Wang et al., 2018). An adult wasp will emerge from the puparia roughly 3-4 weeks after parasitism and live for approximately 4 weeks (Wang et al., 2018). Within 3 days of emergence, females reach full reproductive maturity with the capacity to lay approximately 80 eggs (Wang et al., 2018). The hope for any new parasitoid species for classical biological control is that when established, they will help suppress pest populations either in non-crop habitat or in the crop itself, thereby relieving some of the pressure on growers close to harvest. However, currently it is unknown how far *G. kimorum* is likely to disperse from a release point, which would help in directing the intensity of release efforts aimed at facilitating its establishment. Implementation of parasitoid

wasps as a biocontrol has been shown to help reduce reliance on chemical control by limiting population growth in two lepidopteran pests of corn, *Ostrinia* spp. and *Helicoverpa zea*.

Trichogrammids have been widely used as biocontrol agents for over a century and continue to be implemented against pests of varying orders such as, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Neuroptera (Smith, 1996). Most parasitoid wasps rely on multiple sensory cues to find their hosts (Waage & Hassell, 1982; Vinson S.B., 1976). As a specialist of *D. suzukii*, *G. kimorum* likely uses some combination of host kairomones and fermentation volatiles to find fruit infested by their hosts.

In this study we reared *G. kimorum* and released it at the edge of commercial tart cherry and blueberry plantings, using pre- and post-release sentinel traps and fruit collections to assess baseline larval parasitism and map their ability to find *D. suzukii* larvae under Michigan conditions. The design of this study was heavily influenced by one conducted in Italy studying related species (Rossi-Stacconi et al. 2018). Like the Rossi-Stacconi study, our goal was to better understand the dispersal capacity of *G. kimorum* and to determine whether any evidence of survival could be detected in the same season after the releases.

Materials and methods

Wasp rearing

Parasitoid wasps, *G. kimorum*, were obtained in March 2022 from a USDA-ARS laboratory in Beltsville, MD to establish a colony at Michigan State University (MSU). Early instar *D. suzukii* larvae were exposed to wasps on a blueberry substrate (Rossi-Stacconi et al., 2022). *Drosophila suzukii* were introduced to the blueberries for approximately 48hrs. Flies were supplied from a colony established from infested berries collected at a Michigan farm in 2021 and maintained on a standard solid cornmeal diet (Dalton et al. 2011) in 40 ml polystyrene vials (Genesee

Scientific, CA), in a growth chamber set to $22.8 \pm 2^{\circ}\text{C}$, 65% relative humidity (RH), and a 16h:8 (L:D) photoperiod. Blueberries were purchased from a local supermarket and soaked in DI water three times for 30 min to remove surface residues prior to exposing them to insects. Washed fruit was transferred to 1 gal tubs (1.8L borosilicate glass, 11174000 OXO) and sprinkled with yeast (Fleischmann's Active Dry Yeast) to increase infestation by *D. suzukii* and reduce mold growth. Approximately, 30-50 flies (male and female) were anesthetized with CO_2 and added to the tubs. Tubs were sealed with a fine mesh lid and kept under the same conditions as above for egg laying over a 48-hour duration. After time elapsed, flies were released into a mesh cage, but some were kept to continue egg laying and supplying preferred larval stage for wasps. Wasps were then introduced to each tub of infested fruit in groups of 50 males and 50 females by lightly tapping on their holding vial. Wasps were allowed to parasitize host larvae in the infested fruit for 5 days under the same environmental conditions as *D. suzukii* oviposition. Wasps were aspirated out of tubs within a mesh cage, then incorporated back into the colony to continue egg laying. Infested fruit was kept at normal colony conditions as described above for 3-4 weeks, until adult wasp emergence. Holding vials were prepared by compressing a roll of saturated paper towel into the bottom and adding a drop of honey to the underside of the foam plug. During wasp emergence, adults were aspirated into holding vials in sets of 50 pairs (50 males and 50 females), then inverted and maintained at colony conditions until wasps were needed.

Site selection

This study was conducted at 16 sites across West Michigan in 2023 (Figure 2.1). Locations were chosen based on two main criteria, namely that there was a history of *D. suzukii* populations, and that there was a susceptible crop (tart cherry and highbush blueberry) adjacent to a wooded edge. All seven of the tart cherry sites are commercial operations using conventional

pest management practices. Five of the nine blueberry sites are also managed conventionally, three sites use organic pest management practices, and one site is considered to be unmanaged but is mown and pruned to maintain the bushes and access to the field (Table 2.1).

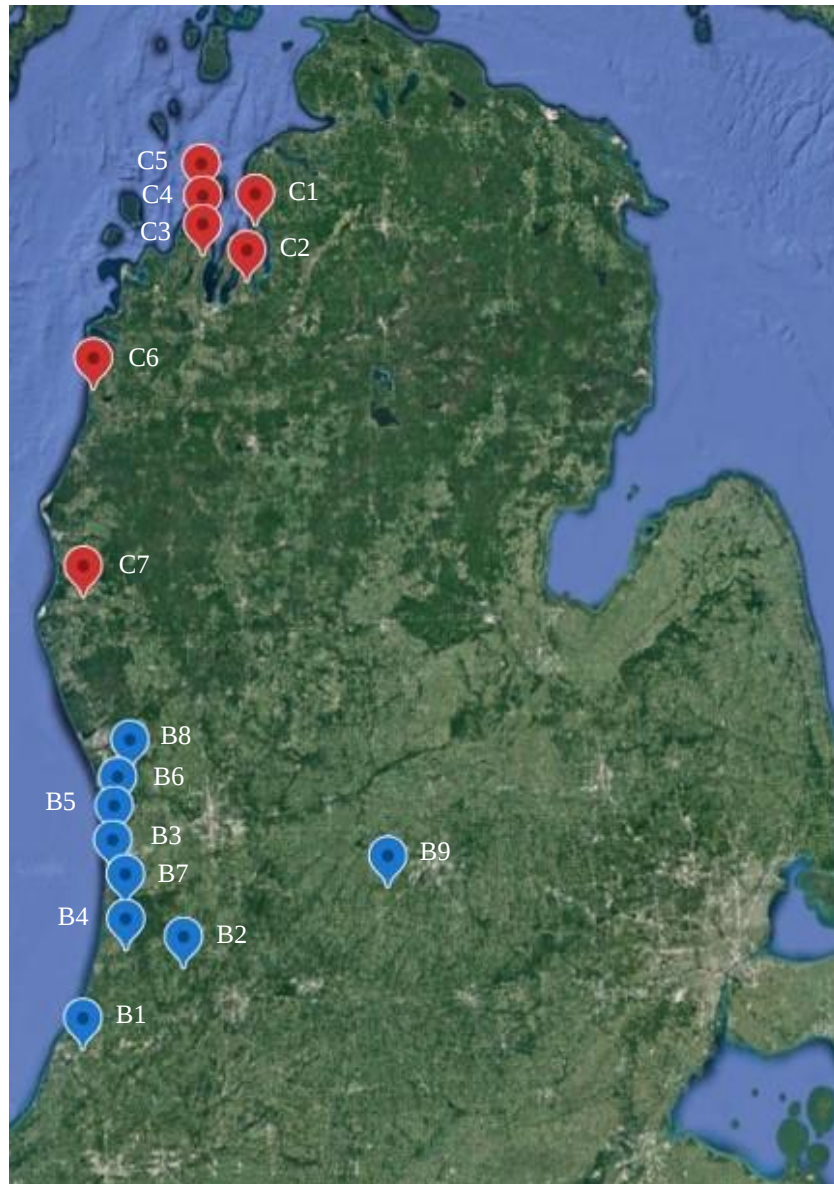


Figure 2.1. Map of blueberry (blue) and tart cherry (red) study sites where *G. kimorum* wasps were released.

Table 2.1. List of study site counties, focal crop, pest management program, and GPS coordinates.

Site ID	County	Crop	Management	Latitude (N)	Longitude (W)
C5	Leelanau	Tart Cherry	Conventional	45.13623	-85.6388
C1	Antrim	Tart Cherry	Conventional	45.02151	-85.3578
C4	Leelanau	Tart Cherry	Organic	45.02123	-85.6342
C3	Leelanau	Tart Cherry	Conventional	44.9193	-85.6384
C2	Grand Traverse	Tart Cherry	Conventional	44.82113	-85.4107
C6	Benzie	Tart Cherry	Conventional	44.45015	-86.2265
C7	Oceana	Tart Cherry	Conventional	43.70789	-86.2997
B8	Muskegon	Blueberry	Conventional	43.08159	-86.0803
B6	Ottawa	Blueberry	Conventional	42.94989	-86.146
B5	Ottawa	Blueberry	Conventional	42.84647	-86.1673
B3	Allegan	Blueberry	Unmanaged	42.72411	-86.1788
B9	Eaton	Blueberry	Organic	42.63763	-84.7906
B7	Allegan	Blueberry	Organic	42.60061	-86.119
B4	Van Buren	Blueberry	Organic	42.43951	-86.1224
B2	Van Buren	Blueberry	Organic	42.37021	-85.8341
B1	Berrien	Blueberry	Unmanaged	42.08491	-86.3497

***Drosophila suzukii* monitoring**

Monitoring of *D. suzukii* populations was conducted all season to track host densities. Weekly activity of *D. suzukii* males and females captured in baited traps hung in the crop canopy near the wooded edge, were used to record relative abundance starting the week of 9 June at tart cherry sites, and the week of 12 July at nearby blueberry sites, through the end of September. All traps were constructed from 32-ounce deli cups with 0.95 cm holes near the lip and baited with an attractant. In the tart cherry orchards, a SCENTRY Spotted Wing *Drosophila* lure (Scentry

Biologicals, MT) was used to attract flies and was suspended over 2 cm of soapy water drowning solution. At the blueberry sites, flies were attracted and captured using a yeast-sugar solution (1 tbsp. of active dry yeast, 4 tbsp. sugar, a drop of unscented dish soap, 12 oz water). Trap contents were collected weekly and returned to the lab to be strained and flies sorted and counted.

Fruit collections

To characterize the resident parasitoid community, fruit samples were collected at all sites using the methods of Abram et al. (2022) and adapted using the approach described by Van Timmeren et al. (in review). At the tart cherry sites, samples were collected on the same day as the first *G. kimorum* release (Table 2.2), when there were still some tart cherries remaining on the trees. On the same dates, the wooded edge of each cherry site was also scouted for wild fruit, and as much fruit, up to a pint per type, was collected where present. Fruit availability later in the season was inadequate for monitoring purposes at tart cherry sites. More intensive fruit sampling was possible at blueberry sites because of their extended ripening period and these sites tended to have more available wild fruit, especially at the unmanaged sites. Fruit sampling from blueberry sites was conducted in wild and crop fruit, during site visitation. Sites that were visited often had sampling done every week, while sites further away had sampling done at pre-release and within one week of releases. Samples were moved to a separate plastic container in lab, each had a mesh top (830 microns) to prevent any parasitoids from escaping during emergence. Fruit was held on a mesh suspended over a saturated substrate of either sponge or cotton pads to regulate humidity. Sticky inserts were placed along the sides of the container to capture emerging insects. All fruit samples were held in colony conditions, as described above, for approximately 6 weeks to allow for insect emergence. Emerged flies were counted and identified to species. Emerged parasitoids from fruit collections were identified to species by sight using modified keys

provided by Matt Buffington (Abram et al., 2022). Parasitoids emerged from sentinel trapping were sent to the Agriculture and Agri-Food Canada, London Research and Development Centre Lab for species identification via PCR (Gariepy et al. 2024)

Table 2.2. Sentinel trapping deployment dates and number of days traps were deployed with respect to *G. kimorum* release.

Site ID	Crop	Starting dates for sentinel trapping (number of days deployed)			
		Pre-release	Release	Post-release #1	Post-release #2
		3 traps/site	9 traps/site	2 traps/site	2 traps/site
		(6-8 days)	(2 days)	(7-8 days)	(7 days)
C5	Tart Cherry	8/1/23	8/8/23	9/21/23	9/29/23
C1	Tart Cherry	7/25/23	8/1/23	9/21/23	9/29/23
C4	Tart Cherry	7/11/23	7/17/23	9/7/23	9/14/23
C3	Tart Cherry	7/17/23	7/25/23	9/14/23	9/21/23
C2	Tart Cherry	7/17/23	7/25/23	9/14/23	9/21/23
C6	Tart Cherry	7/18/23	7/25/23	9/14/23	9/21/23
C7	Tart Cherry	7/11/23	7/18/23	9/7/23	9/14/23
B8	Blueberry	8/23/23	8/31/23	9/26/23	10/3/23
B6	Blueberry	8/23/23	8/31/23	9/26/23	10/3/23
B5	Blueberry	8/16/23	8/23/23	9/19/23	9/26/23
B3	Blueberry	8/9/23	8/16/23	9/12/23	9/19/23
B9	Blueberry	8/16/23	8/23/23	9/19/23	9/26/23
B7	Blueberry	8/16/23	8/23/23	9/19/23	9/26/23
B4	Blueberry	8/9/23	8/16/23	9/12/23	9/19/23
B2	Blueberry	8/9/23	8/16/23	9/12/23	9/19/23
B1	Blueberry	8/9/23	8/16/23	9/12/23	9/19/23

***Ganaspis kimorum* releases**

At each release event, two containers, each containing approximately 500 wasps with a 50:50 sex ratio, were used to transport and release 1,000 *G. kimorum*, for a total of 23,000 wasps (14 tart cherry and 9 blueberry releases). Release containers were constructed from 32-oz deli cups modified with 3 foam plugs for air exchange (Figure 2.2C). Honey was provided *ad libitum* and a piece of paper toweling on the lid end was used for humidity control. Release containers were transported to field sites in coolers, and upon arrival, inserted into plastic delta traps with the lid end facing out, and the trap hung in the wooded edge at all sites except for GB3, which was set within the crop to prevent disturbance at this public-access park. The lid was removed when it was time to release the wasps; wasps were allowed to exit on their own, or the container gently tapped to persuade stragglers. Two releases were made at each tart cherry site with the first release timed to occur immediately after harvest (Table 2.2). The second tart cherry release occurred 21-22 days later at all but site E1, which occurred 14 days after the first-round release. A single release was conducted at each of the nine blueberry sites (Table 2.2), also timed with post-harvest. Post-harvest timing for releases was chosen to minimize insecticide exposure, and to maximize available larvae in fruit that remained.

Sentinel trapping

Sentinel traps were deployed four times at each site to sample resident larval parasitoids able to infest *D. suzukii* and as a means to detect *G. kimorum* populations after releases (Table 2.2). Sentinel traps (Figure 2.2C) consisted of an orange delta trap containing two 12-oz deli-cup inserts with lids modified with a piece of fine mesh (830 microns) to prevent drosophilid movement while allowing parasitoids to freely pass through (Huang et al. 2023). Each insert was prepared with a piece of cellulose sponge and a layer of paper toweling to soak up excess

moisture. Each insert was supplied with one of two pre-infested fruit baits (blueberry and banana). Store-bought blueberries were prepared as described above, and a single layer of blueberries added on top of the paper toweling. With the peel still intact, bananas were cut lengthwise and then in half, with one piece used per insert. Fruit baits were infested with *D. suzukii* (50 pairs male and female flies per arena) for 48 hours, to obtain 2nd instar larvae. Trap inserts were transported to the field in a cooler.

Traps were located in relation to the intended release point at each site, along the wooded edge at all four time points, and into the crop at the first and second time points. Three traps, each 10 m from the release point, were set up one week before the first releases at each site, with two along the wooded edge and the third trap into the crop (Figure 2.2A). Between 6-8 days later, inserts were collected and replaced, and 6 additional traps were added to each site, on the same day as and just prior to the first wasp release. Distances were based on a dispersal study of a *D. suzukii* pupal parasitoid in Italy (Rossi-Stacconi et al. 2018). In cherry orchards, traps were added at 20 and 40 m (Figure 2.2B), however, due to field size limitations, the additional sentinel traps at blueberry sites were set up at 5 and 20 m (Figure 2.2C) from the wasp release points. Traps deployed just before releases were collected after 48 hours. The third sentinel trapping event, which consisted of two traps in the wooded edge, 10 m away from the release point, occurred 26-30 days after the last wasp release as a means to intercept 2nd generation *G. kimorum*. Inserts were collected 7-8 days later and replaced with the final round of inserts, collected 7 days after that (Table 2.2). Inserts were transported back to the lab in a cooler and placed under controlled environmental conditions for 6 weeks to allow any parasitoids to emerge. Emerged wasps were collected and sent to the Agriculture and Agri-Food Canada,

London Research and Development Centre Lab for species identification via PCR (Garipey et al. 2024).

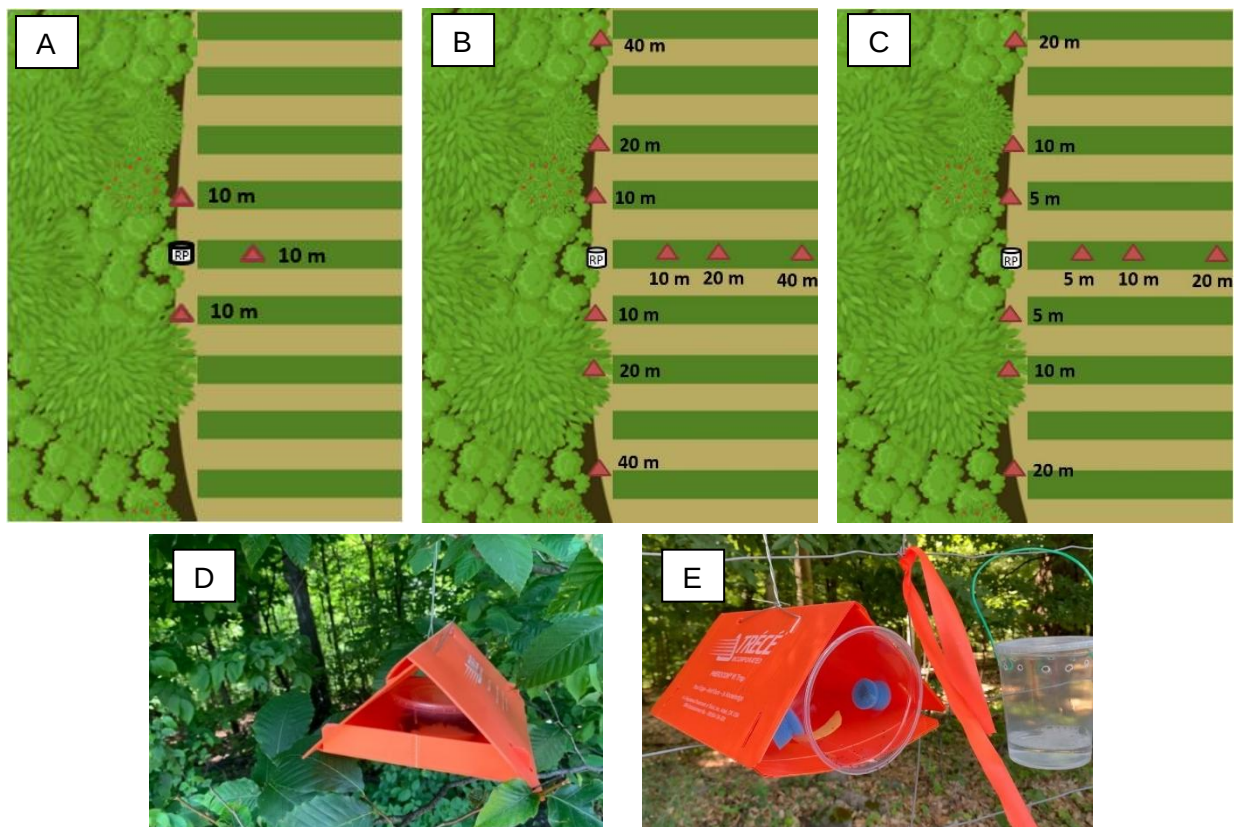


Figure 2.2. Diagrams of sentinel trap placement and images of traps used to assess baseline parasitoid activity pre-release, measure dispersal post release, and monitor *D. suzukii* populations. At pre-release, traps were set at 10m at each transect to establish a baseline of zero *G. kimorum* at each site (A). Post-release trapping at cherry sites had traps set at 10m, 20m, and 40m from the release point (B). Post-release trapping at blueberry sites had traps set at 5m, 10m, 20m to accommodate smaller field sizes (C). Sentinel traps used to monitor parasitoid activity were constructed from an orange corrugated plastic delta trap baited with two 8 oz deli cups with mesh lids containing either pre-infested blueberries or bananas (D). Orange delta traps were also used to shelter 32-oz deli cups from which wasps were released.

Results

No second-generation *G. kimorum* emerged from sentinel traps, but a total of 63 individuals emerged from fruit collected within 2 days of the releases at 5 out of the 16 sites, all of which were blueberry sites (Figure 2.3, Table 2.3). Of these specimens, 81% emerged from a single organic site. An adventive larval parasitoid species that also attacks *D. suzukii*, *Leptopilina japonica* (Novković & Kimura) (Hymenoptera: Figitidae), was recorded emerging from sentinel traps and/or fruit collections from 11 of the 16 sites (Table 2.3), all but one of which were blueberry sites. Blueberry sites had the highest proportion of parasitoids at 99.33% of total parasitoids collected and *Leptopilina japonica* was by far the most abundant parasitoid of the two species comprising approximately 94% of the total parasitoids collected (Figure 2.4). Only two of the tart cherry release sites had *L. japonica* detections (Figure 2.3B, Table 2.3).

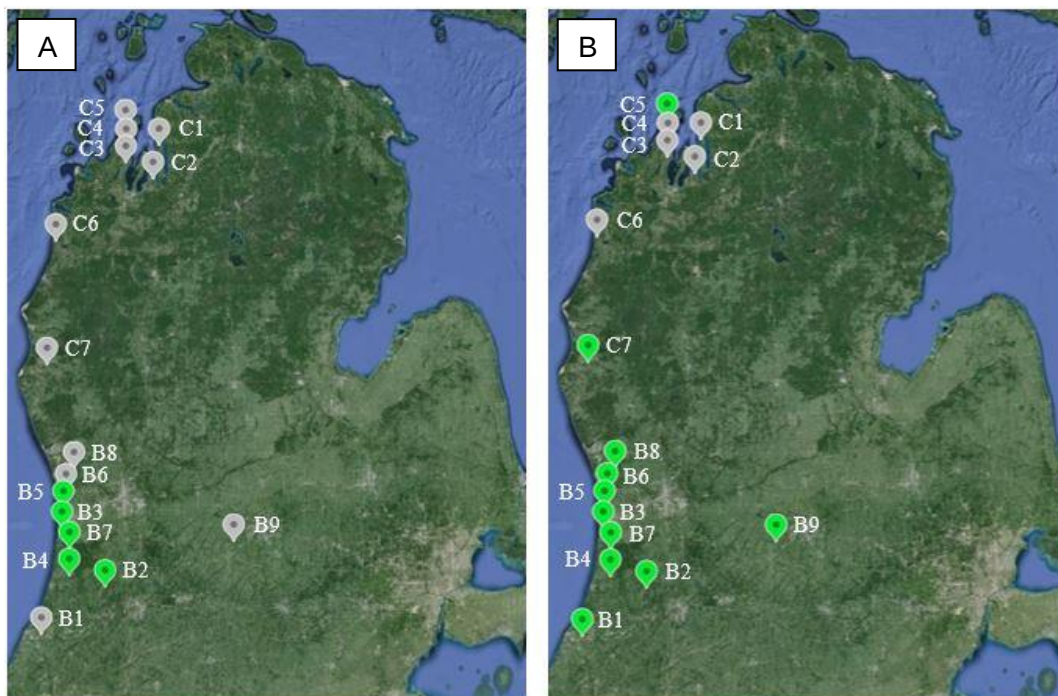


Figure 2.3. Map of study sites in Michigan with green denoting sites where *G. kimorum* (A) and of *L. japonica* (B) were detected in samples.

No *G. kimorum* populations were detected at any of the sites prior to their release in 2023. Detection of parasitoids increased throughout the season, yielding the highest number of emerged wasps at the end of the season post-harvest. Retrieved traps from tart cherry orchards yielded a total of 7 *L. japonica* wasps from conventional orchards, after harvest. Sentinel traps in blueberry had the highest abundance of *L. japonica* emerge from organic sites overall, unmanaged and conventional systems had a similar number of wasps emerge. In fruit collections, *G. kimorum* was detected at 5 of the 9 blueberry sites post-release. Organic sites yielded the greatest numbers of *L. japonica* and *G. kimorum*, with unmanaged sites having the second most abundance. Populations measured from fruit collections were at the highest post-harvest, later in the season. Similar to sentinel trapping, fruit collections yielded a similar number of wasps in the southern sites compared to the northern sites.

Drosophila suzukii traps at tart cherry sites revealed peak population levels in late August and early September, nearly a month after *G. kimorum* releases occurred, with a sharp decline before post-release sampling began (Figure 2.6). In blueberry, *G. kimorum* releases coincided with rising *D. suzukii* populations and were still rising when post-release sampling occurred (Figure 2.6).

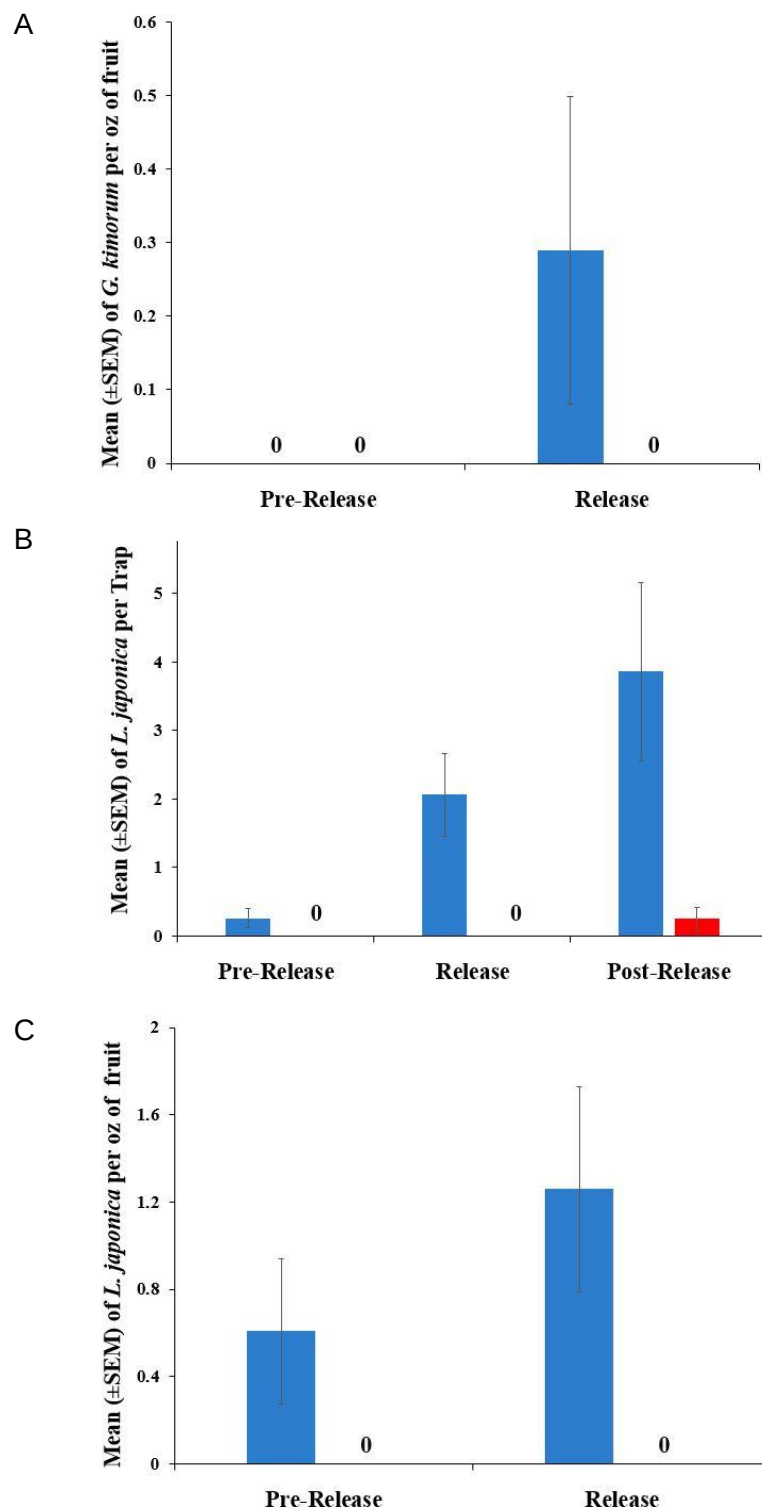


Figure 2.4. Comparison of tart cherry orchards and highbush blueberry for mean *G. kimorum* per ounce of fruit collected (A), the mean number of *L. japonica* emerged from sentinel traps (B), and mean *L. japonica* per ounce of fruit collected (C).

Table 2.3. Total number of larval parasitoids of *D. suzukii* that emerged from either sentinel traps or fruit collections at each site.

Site ID	Crop Type	IPM Program	<i>G. kimorum</i>		<i>L. japonica</i>	
			Sentinel trap	Fruit collections	Sentinel trap	Fruit collections
CH5	Tart Cherry	Conventional	0	0	3	0
CH1	Tart Cherry	Conventional	0	0	0	0
CH4	Tart Cherry	Organic	0	0	0	0
CH3	Tart Cherry	Conventional	0	0	0	0
CH2	Tart Cherry	Conventional	0	0	0	0
CH6	Tart Cherry	Conventional	0	0	0	0
CH7	Tart Cherry	Conventional	0	0	4	0
BB8	Blueberry	Conventional	0	0	11	10
BB6	Blueberry	Conventional	0	0	2	9
BB5	Blueberry	Conventional	0	1	20	13
BB3	Blueberry	Unmanaged	0	1	42	333
BB9	Blueberry	Organic	0	0	108	55
BB7	Blueberry	Organic	0	9	56	206
BB4	Blueberry	Organic	0	51	22	25
BB2	Blueberry	Organic	0	1	22	11
BB1	Blueberry	Unmanaged	0	0	25	8
Total			0	63	315	670

Most of the parasitoids from fruit collections emerged from either cultivated blueberries, wild blackberries or wild cherry collected from the wooded edges adjacent to blueberry sites; wild ripe fruit was scarce in wooded edges adjacent to the cherry sites (Figure 2.7). *Ganaspis kimorum* only emerged from cultivated blueberry or wild blackberry adjacent to cultivated blueberry (Figure 2.7D). Neither species emerged from pokeweed, honeysuckle, elderberry, or cultivated tart cherry (Figure 2.7).

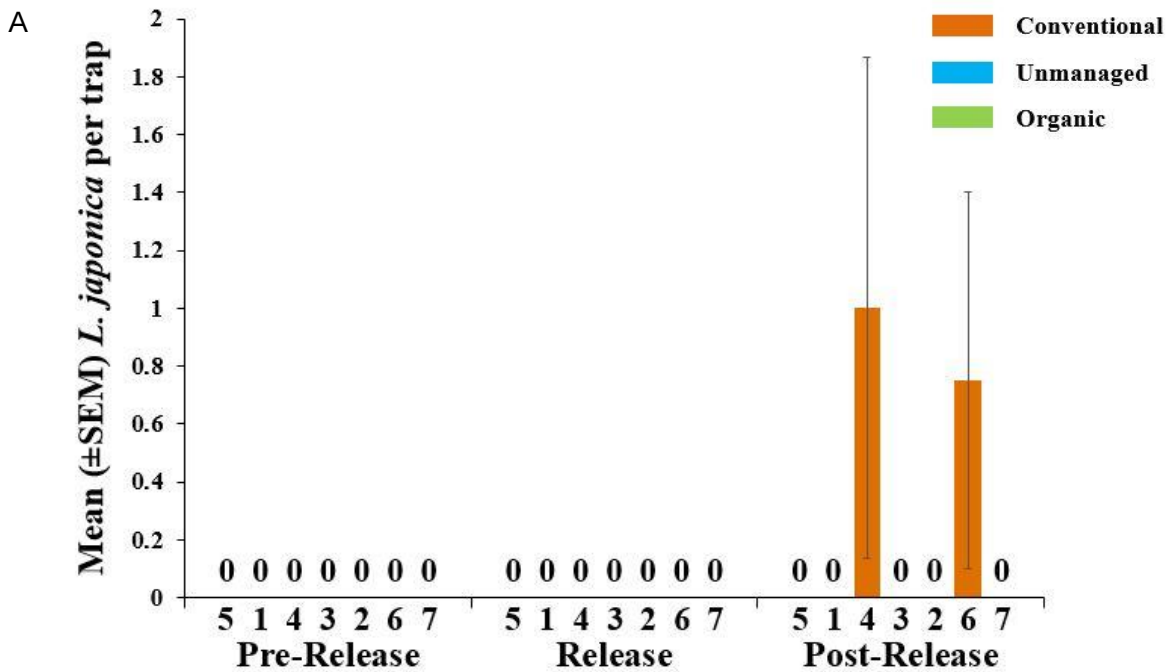


Figure 2.5. Number of *L. japonica* caught per sentinel trap with respect to the release of *G. kimorum* at each tart cherry (A) and blueberry (B) site. Pre-release and post-release traps were deployed for 6-8 days; release traps were deployed for two days. Mean number of *G. kimorum* that emerged from wild and cultivated fruit collections at nine blueberry sites with respect to the *G. kimorum* releases (C). Mean number of *L. japonica* that emerged from wild and cultivated fruit collections at nine blueberry sites with respect to the *G. kimorum* releases (D).

Figure 2.5. (cont'd)

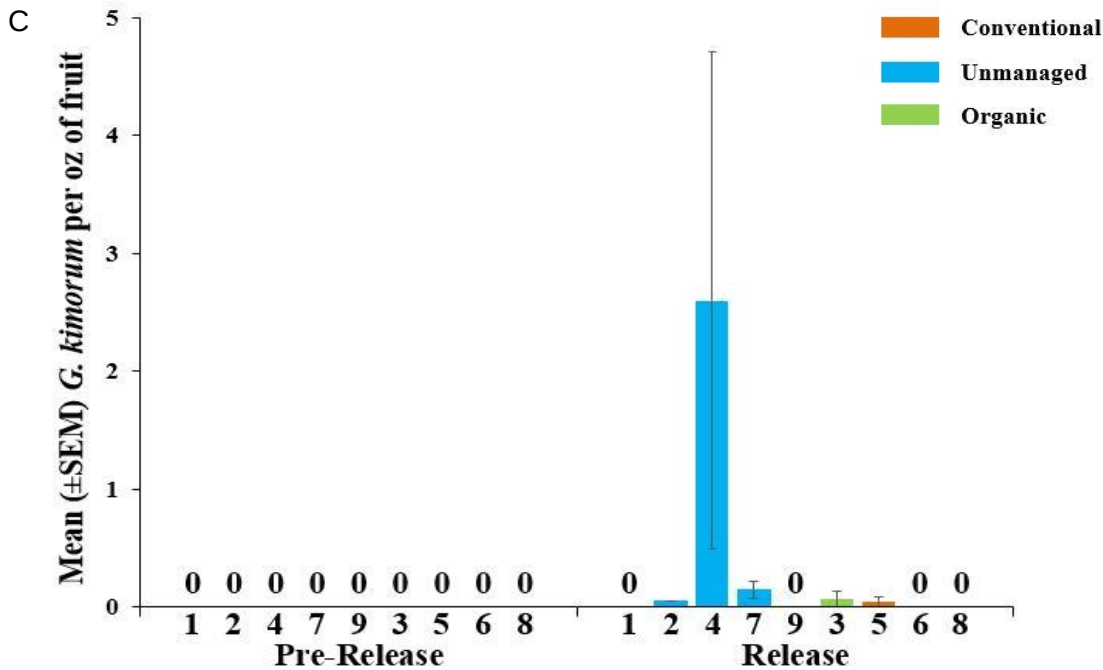
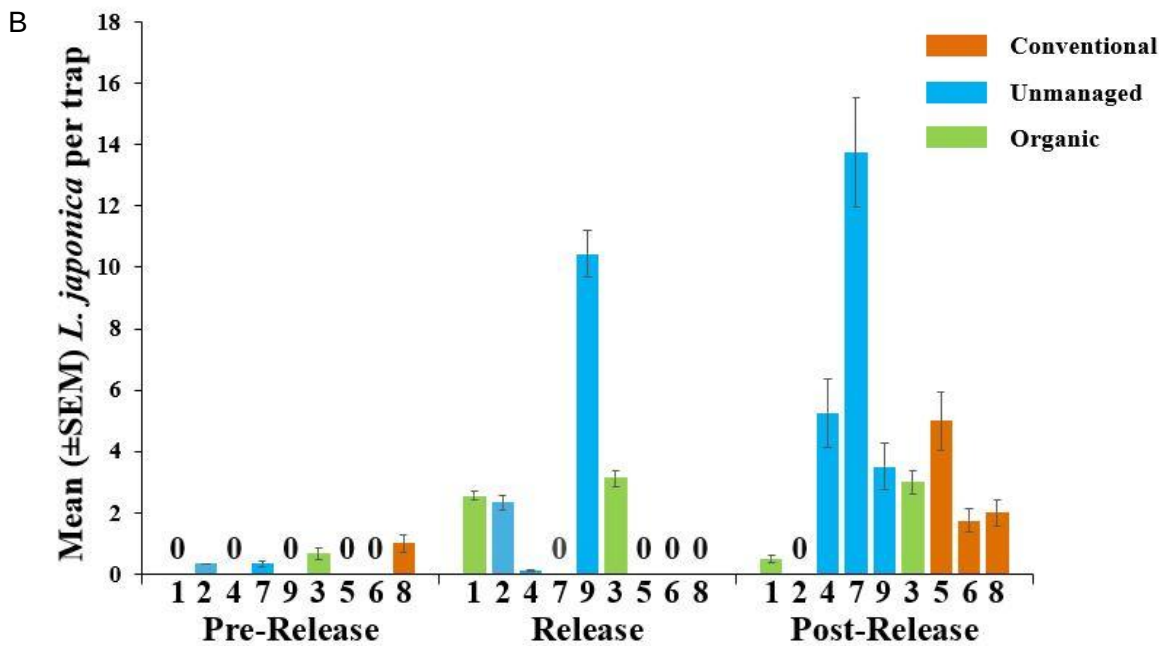
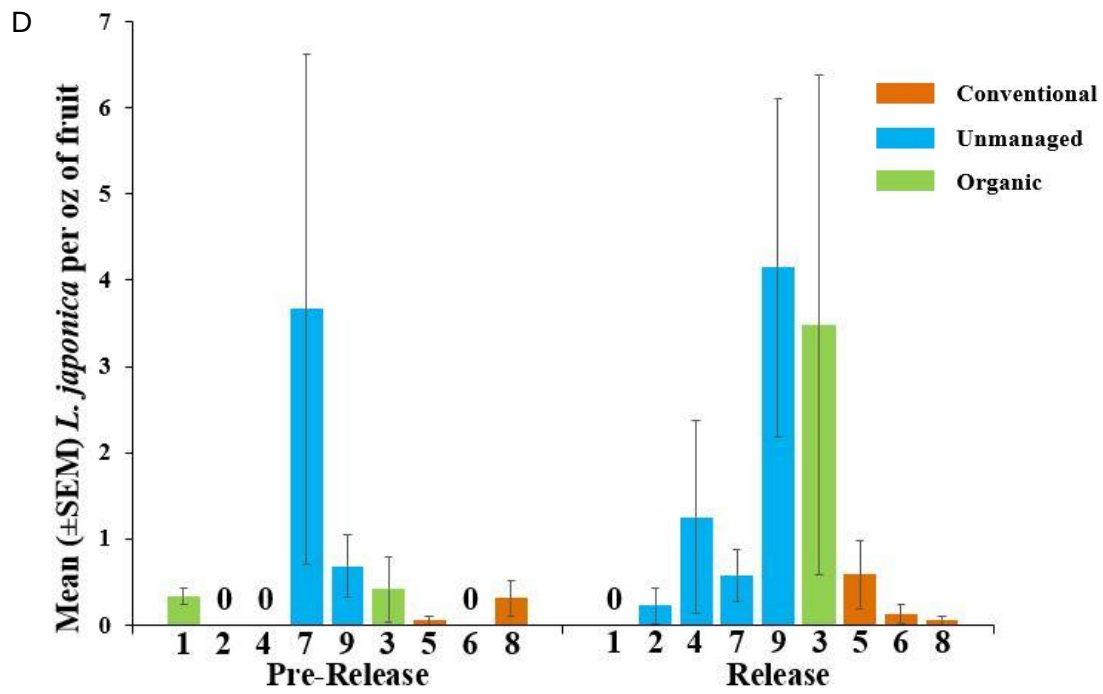


Figure 2.5. (cont'd)



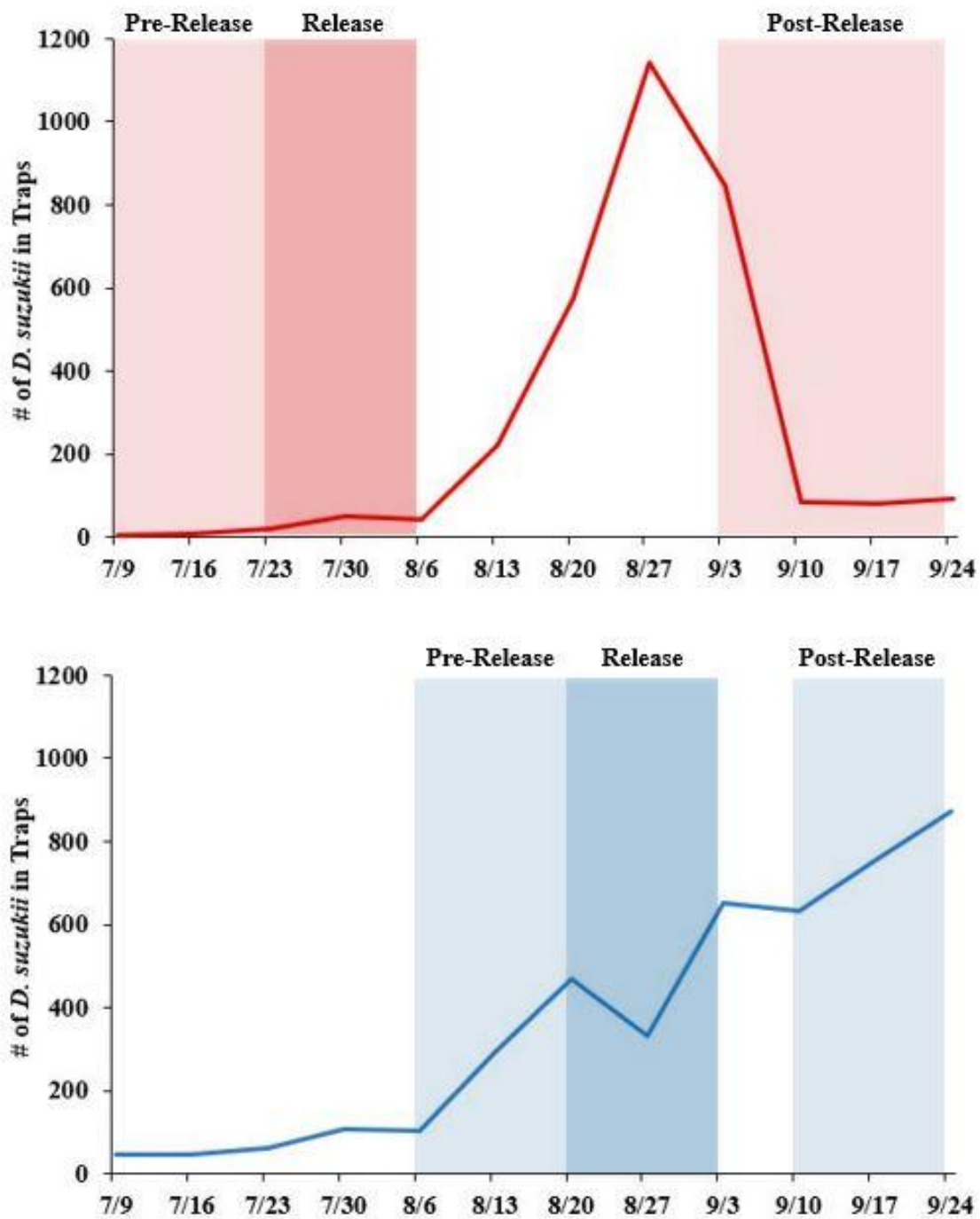


Figure 2.6. Mean number of *D. suzukii* captured by scentry traps (tart cherry, red lines) or yeast traps (blueberry, blue lines) from July 9 to September 24, 2023. Tart cherry sites received 1 trap at 9 sites, while blueberry received one trap at 3 sites and 2 traps at 1 site (B7). Traps were processed and exchanged weekly.

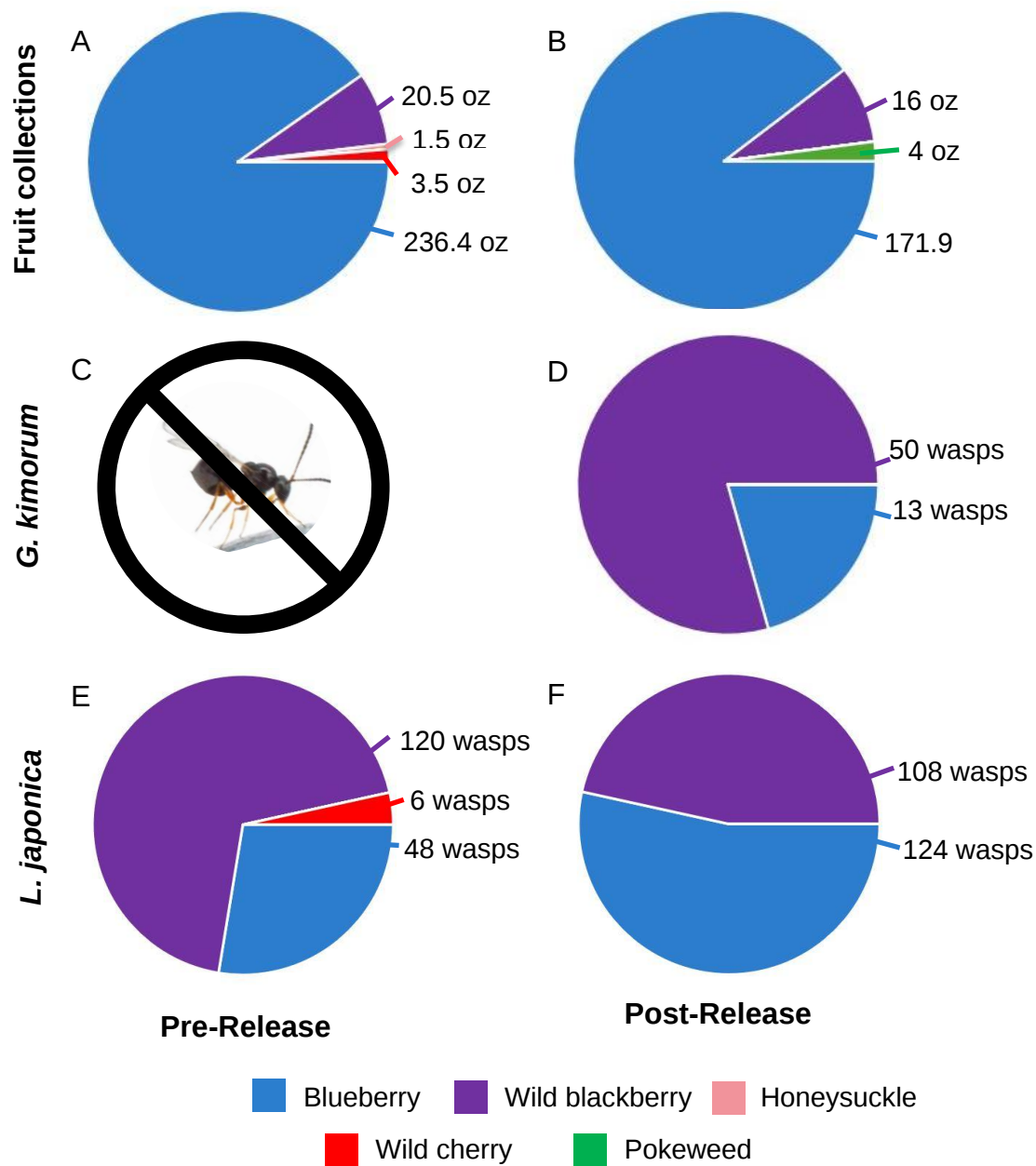


Figure 2.7. Proportion of fruit collected by volume prior to release (A) and post- release (B). Number of emerged *G. kimorum* from fruit samples by fruit type prior to their release (C) and post-release (D). Number of *L. japonica* that emerged from fruit collections by fruit type prior to *G. kimorum* release (E) and post-release (F).

Discussion

In this study we set out to rear, release, and assess the dispersal capacity of a selective biological control agent that targets *Drosophila suzukii*, a key invasive pest of cultivated blueberry and tart cherry. This study provides the first evidence of reproduction on *D. suzukii* under field conditions in Michigan. In addition, we documented the establishment of a second, less-selective larval parasitoid species, *Leptopilina japonica* which seems to be established at field sites regardless of management program. This suggests that this form of biological control against *D. suzukii* is compatible with Michigan landscapes.

Pest management practices impact agricultural landscapes, improving or creating an unfavorable habitat for natural enemies. Disturbances from management practices can influence potential establishment of parasitoids and can be detrimental to their success (Landis et al., 2000). While organic agricultural methods are typically characterized by the incorporation of cultural or other non-chemical tools compared with conventional systems, practices that are associated with the conservation of local biodiversity and the provision of refugia for natural enemies (Bengtsson et al., 2005), both conventional and organic production systems where *D. suzukii* is a pest rely on broad spectrum insecticides to protect fruit from infestation (Beers et al., 2011). This intensive use of insecticides increases the potential mortality of beneficial insects that could provide natural pest suppression (Beers et al., 2011). Unmanaged sites at which no pesticides are applied are hypothesized to allow for maximum conservation of biodiversity and associated ecosystem services, providing resources for natural enemies to thrive (Landis et al., 2000), but this strategy results in low yields and fruit losses to pests that are incompatible with the economic realities of commercial fruit production. In this study, releases of *G. kimorum* were timed to coincide with post-harvest after insecticide use had ceased for the season, a decision

intended to minimize insecticide exposure and improve establishment success. However, *L. japonica* experienced normal management practices throughout the season, and still was detected at all the blueberry sites regardless of the underlying pest management program. This suggests that the surrounding habitat typical of Michigan fruit production areas can act as a reservoir and support parasitoids that attack *D. suzukii*.

Sites under organic pest management yielded the highest parasitoid emergence compared to those managed conventionally or minimally managed. Lower parasitoid populations at our unmanaged sites could be from their size and high human activity, reducing the amount of natural growth surrounding the area. One site (B1) is a low-input, small planting at a research station and another site (B3) has public access with encouraged “U-Pick” decreasing available fruit resources at the site. In contrast, our organic sites contained larger contiguous plantings with multiple cultivars, adjacent to large parcels of unmanaged land that contained abundant wild host fruit attractive to *D. suzukii*, ostensibly increasing the supply of hosts to *L. japonica* and *G. kimorum*.

Leptopilina japonica is an adventive natural enemy of *D. suzukii*, first detected in 2022 in Michigan (Garipey et al., 2024). It was originally considered to be a biocontrol agent for release but had too wide of a host range, able to parasitize both *D. melanogaster* (Meigen) (Diptera: Drosophilidae) and *D. suzukii* (Girod et al., 2018). As a generalist, *L. japonica* is able to outcompete other parasitoids and begin growing populations earlier in the season. *Leptopilina japonica* does not discriminate against larvae already parasitized by *G. kimorum* and has a faster development time (Wang et al., 2019). This benefits *L. japonica* by allowing its larvae to attack a *G. kimorum* egg or larvae and take over the host. *Leptopilina japonica* had the largest proportion of emergence from traps and fruit collections in blueberries. Its population growth coincided

with that of *D. suzukii* and was detected more than 2 weeks before *G. kimorum*. It may be that in our sentinel traps larvae previously parasitized by *G. kimorum* were then parasitized by *L. japonica*, as host populations became limited. In a study conducted by Fellin in northern Italy, release of *Ganaspis kimorum* was done with sampling of fresh and fallen fruit to determine interactions on non-target species and impact on *D. suzukii*. Similar to our own observations, the study reported a low recapture of *G. kimorum* across all release sites, with a large percentage of recapture at a single site. Of the samples collected, they found that populations of *L. japonica* were also abundant in areas where *D. suzukii* populations were high (Fellin et al., 2023).

In 2023, tart cherry sites had little to no wild fruit available in wooded edges due to a period of drought in late spring. A single tart cherry cultivar, Montmorency, dominates cherry production systems in Michigan because it exhibits uniform ripening and can be mechanically harvested. In a commercial tart cherry orchard, fruit ripening occurs over a 3–4 week span, and a month earlier than most blueberry cultivars, which means the window for infestation by *D. suzukii* is much narrower than in blueberry. In seasons like 2023, when fruit development is accelerated by an early spring and mid-season heat waves, fewer susceptible fruit will overlap with the surge in *D. suzukii*. Since our releases and surveys were conducted relative to harvest, there was an asynchronous timing between *D. suzukii* populations and monitoring events (Figure 3.6). Infestation rates were lower than previous years in tart cherry orchards and *D. suzukii* population density likely limited the parasitoids' resources for reproduction. While this is only the second year that *L. japonica* has been detected in tart cherries in Michigan orchards, *Ganaspis kimorum* has yet to be detected; lack of commercial fruit and wild hosts in tart cherry farms in West Central and NW Michigan may explain the lack of detection. It would be good to

repeat this study during a season when fruit ripening and pest pressure from *D. suzukii* more closely aligns in tart cherry orchards.

Foraging behavior in parasitoids has been studied to better understand infested plant-volatile cues that are used to find their hosts, and a lack of infested fruit can induce parasitoids to disperse to more suitable habitats (Vosteen et al., 2020). In a study conducted on parasitoids of cereal leaf beetle and alfalfa weevils reviewed by Evans (2018), specialized natural enemies readily moved large distances to find their hosts. Tart cherry orchards have a short time interval of available fruit from development to harvest and if adjacent habitats also lack an abundance of wild fruit, this would make available resources fragmented. In the case of *G. kimorum*, their foraging costs may have outweighed the lack of host availability, dispersing beyond our traps or into the tree lines in pursuit of more readily available hosts. While in blueberry fields, most wasps were found in wild fruit in tree lines rather than available crop fruit. This may display that *G. kimorum* has a preference for certain oviposition substrates when identifying hosts.

In blueberry fields, parasitoid activity was far easier to detect in comparison to tart cherry orchards. Highbush blueberries develop fruit later in the season than tart cherries, and within an individual bush fruit do not all ripen at the same time. Within a farm, it is typical for growers to have early to late ripening cultivars to maximize fruit availability for fresh markets. This results in an abundance of ripening fruit over a longer period of time than in tart cherry systems, and a time frame that typically aligns with the phenology of *D. suzukii* (Garcia-Salazar, 2002, Grassi et al., 2018). Blueberries thrive in sandy acidic soils, conditions which are also well-suited to producing wild small berry fruit in adjacent non-crop habitat, which attract *D. suzukii* populations (Rodriguez-Saona et al., 2019). The practical consequence is that *D. suzukii* have ample resources to reproduce and build populations throughout the season, both when the crop is

not yet at a susceptible stage and afterwards for many weeks. This abundance of wild and cultivated fruit is expected to provide an abundance of reproductive resources to the parasitoids and increases their chances of establishment (Landis et al., 2000, Lee et al., 2015).

Fruit sampling was generally superior to sentinel traps for revealing parasitoid activity. Fruit collections had the greatest yield of parasitoids in the season, specifically wild blackberry fruit. Fruit collected after the *G. kimorum* releases, when *D. suzukii* populations were higher, had the largest proportion of wasps emerge compared to pre-release collections. Since releases were timed approximately around the time of harvest, cultivated fruit availability was limited, and wild fruit would attract greater host numbers for parasitoids to target. Populations found in wild fruit after harvest suggests that parasitism is taking place in non-crop habitats when food resources are low. Successful targeting by parasitoids, *L. japonica* and *G. kimorum*, in non-crop habitat would increase their potential for establishment success, by following hosts onto varying substrates. However, fruit collections became limited in post-release after harvest as much of the cultivated fruit had been removed, reducing the amount of available fruit resources to their host in the late season. Sentinel trapping may be the best strategy late in the season for detecting presence of parasitoids, while fruit collections offer a more accurate picture of population densities, fruit baits are more attractive to hosts post-harvest when fruit resources are low (Pelton et al., 2016, Urbaneja-Bernat et al., 2020). Overall, rearing wasps from sentinel traps and fruit collections is limited in their assessment of population densities since only a proportion of wasps are successful in their development. Further research is needed to identify trapping mechanisms to capture or measure live adults to accurately estimate population densities of *L. japonica* and *G. kimorum* in crop habitat and non-crop wild areas.

LITERATURE CITED

- Abram, P. K., McPherson, A. E., Kula, R., Hueppelsheuser, T., Thiessen, J., Perlman, S. J., Curtis, C. I., Fraser, J. L., Tam, J., Carrillo, J., Gates, M., Scheffer, S., Lewis, M., & Buffington, M. (2020). New records of Leptopilina, Ganaspis, and Asobara species associated with *Drosophila suzukii* in North America, including detections of *L. japonica* and *G. brasiliensis*. *Journal of Hymenoptera Research*, 78, 1–17.
- Abram, P.K., Wang, X., Hueppelsheuser, T., Franklin, M.T., Daane, K.M., Lee, J.C., Lue, C.-H., Girod, P., Carrillo, J., Wong, W.H.L., Kula, R.R., Gates, M.W., Hogg, B.N., Moffat, C.E., Hoelmer, K.A., Sial, A.A., Buffington, M.L. (2022) A coordinated sampling and identification methodology for larval parasitoids of spotted-wing drosophila. *Journal of Economic Entomology*, 115(4): 922–942.
- Asplen, M. K., Anfora, G., Biondi, A., Choi, D. S., Chu, D., Daane, K. M., Gilbert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Véték, G., Vogt, H., Walton, V.M., Yu, Y., Zappalá, L., & Desneux, N. (2015). Invasion biology of spotted wing drosophila (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*, 88, 469-494.
- Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., & Kopp, A. (2014). The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society B: Biological Sciences*, 281.
- Beers, E. H., Van Steenwyk, R. A., Shearer, P. W., Coates, W. W., & Grant, J. A. (2011). Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. *Pest Management Science*, 67(11), 1386-1395.
- Bengtsson, J., Ahnström, J., & Weibull, A. C. (2005). The effects of organic agriculture on biodiversity and abundance: a meta-analysis. *Journal of Applied Ecology*, 42(2), 261-269.
- Bruck, D. J., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., DeFrancesco, J., Gerdeman, B., & Spitler, H. (2011). Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. *Pest Management Science*, 67(11), 1375-1385.
- Daane, K. M., Wang, X. G., Biondi, A., Miller, B., Miller, J. C., Riedl, H., Shearer, P.W., Guerrieri, E., Giorgini, M., Buffington, M., Achterberg, K.V., Song, Y., Kang, T., Yi, H., Jung, C., Lee, D.W., Chung, B.K., Hoelmer, K.A., & Walton, V. M. (2016). First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents. *Journal of Pest Science*, 89, 823-835.
- Dalton DT, Walton VM, Shearer PW, Walsh DB, Caprile J, Isaacs R (2011) Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. *Pest Management Science* 67(11): 1368–1374.

- Evans, E. W. (2018). Dispersal in host–parasitoid interactions: Crop colonization by pests and specialist enemies. *Insects*, 9(4), 134.
- Fellin, L., A. Grassi, S. Puppato, A. Saddi, G. Anfora, C. Ioriatti, and M.V. Rossi-Stacconi (2023) First report on classical biological control releases of the larval parasitoid *Ganaspis brasiliensis* against *Drosophila suzukii* in northern Italy. *BioControl* 68: 1–12, <https://doi.org/10.1007/s10526-022-10174-2>
- Garcia-Salazar, C. (2002). Crop timeline for blueberries in Michigan and Indiana. *Prepared for the US Environmental Protection Agency*.
- Gariepy, T.D., P.K. Abram, C. Adams, D. Beal, E. Beers, J. Beetle, D. Biddinger, G. Brind'Amour, A. Bruin, M. Buffington, H. Burrack, K.M. Daane, K. Demchak, P. Fanning, A. Gillett, K. Hamby, K. Hoelmer, B. Hogg, R. Isaacs, B. Johnson, J.C. Lee, H.K. Levenson, G. Loeb, A. Lovero, J.M. Milnes, K.R. Park, P. Prade, K. Regan, J.M. Renkema, C. Rodriguez-Saona, S. Neupane, C. Jones, A. Sial, P. Smytheman, A. Stout, S. Van Timmeren, V.M. Walton, J.K. Wilson, X. Wang (2024) Adventive establishment of *Leptopilina japonica* (Hymenoptera: Figitidae) in North America and development of a multiplex PCR assay to identify key parasitoids of *Drosophila suzukii* (Diptera: Drosophilidae). *NeoBiota*, 93: 63-90.
- Giorgini, M., Wang, X. G., Wang, Y., Chen, F. S., Hougardy, E., Zhang, H. M., Chen, Z.Q., Chen, H.Y., Liu, C.X., Cascone, P., Formisano, G., Carvalho, G.A., Biondi, A., Buffington, M., Daane, K.M., Hoelmer, K.A., & Guerrieri, E. (2019). Exploration for native parasitoids of *Drosophila suzukii* in China reveals a diversity of parasitoid species and narrow host range of the dominant parasitoid. *Journal of Pest Science*, 92, 509-522.
- Girod, P., Lierhmann, O., Urvois, T., Turlings, T. C., Kenis, M., & Haye, T. (2018). Host specificity of Asian parasitoids for potential classical biological control of *Drosophila suzukii*. *Journal of Pest Science*, 91, 1241-1250.
- Grassi, A., Gottardello, A., Dalton, D. T., Tait, G., Rendon, D., Ioriatti, C., Gibeaut, D., Rossi-Stacconi, M.V., & Walton, V. M. (2018). Seasonal reproductive biology of *Drosophila suzukii* (Diptera: Drosophilidae) in temperate climates. *Environmental Entomology*, 47(1), 166-174.
- Gress, B. E., & Zalom, F. G. (2019). Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. *Pest management science*, 75(5), 1270-1276.
- Huang, J., Leach, H., Buffington, M., Rothwell, N., and Wilson, J.K. (2023) Resident parasitoids associated with Drosophilidae in Michigan tart cherry orchards and woodland edges. *Journal of Hymenoptera Research*, 96: 485–494.

- Kacsoh, B. Z., & Schlenke, T. A. (2012). High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. *PloS One*, 7(4), e34721.
- Kanzawa, T. (1939). Studies on *Drosophila suzukii* Mats. Kofu, Yamanashi Agricultural Experiment Station, 49 pp. *Review of Applied Entomology*, 29, 622 (abst.).
- Haye, T., Girod, P., Cuthbertson, A. G. S., Wang, X. G., Daane, K. M., Hoelmer, K. A., Baroffio, C., Zhang, J.P., & Desneux, N. (2016). Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. *Journal of Pest Science*, 89, 643-651.
- Landis, D. A., Wratten, S. D., & Gurr, G. M. (2000). Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*, 45(1), 175-201.
- Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Van Steenwyk, R. A., & Yorgey, B. M. (2011). The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Management Science*, 67(11), 1358–1367.
- Lee, J. C., Dreves, A. J., Cave, A. M., Kawai, S., Isaacs, R., Miller, J. C., Van Timmeren, S., & Bruck, D. J. (2015). Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). *Annals of the Entomological Society of America*, 108(2), 117–129.
- Lee, J. C., Wang, X., Daane, K. M., Hoelmer, K. A., Isaacs, R., Sial, A. A., & Walton, V. M. (2019). Biological control of Spotted-Wing *Drosophila* (Diptera: Drosophilidae) current and pending tactics. *Journal of Integrated Pest Management*, 10(1).
- Mitsui, H., Takahashi, K. H., & Kimura, M. T. (2006). Spatial distributions and clutch sizes of *Drosophila* species ovipositing on cherry fruits of different stages. *Population Ecology*, 48(3), 233-237.
- Nomano, F. Y., Kasuya, N., Matsuura, A., Suwito, A., Mitsui, H., Buffington, M. L., & Kimura, M. T. (2017). Genetic differentiation of *Ganaspis brasiliensis* (Hymenoptera: figitidae) from East and Southeast Asia. *Applied Entomology and Zoology*, 52, 429-437.
- Pelton, E., Gratton, C., Isaacs, R., Van Timmeren, S., Blanton, A., & Guédot, C. (2016). Earlier activity of *Drosophila suzukii* in high woodland landscapes but relative abundance is unaffected. *Journal of Pest Science*, 89, 725-733.
- Rossi-Stacconi, M.V., Amiresmaeili, N., Biondi, A., Carli, C., Caruso, S., Dindo, M.L., Francati, S., Gottardello, A., Grassi, A., Lupi, D., Marchetti, E., Mazzetto, F., Mori, N., Pantezzi, T., Tavella, L., Garzia, G.T., Tonina, L., Vaccari, G., Anfora, G., and Ioriatti, C. (2018) Host location and dispersal ability of the cosmopolitan parasitoid *Trichopria drosophilae* released to control the invasive spotted wing *Drosophila*. *Biological Control*, 117: 188-196.

- Rossi-Stacconi, M. V., Wang, X., Stout, A., Fellin, L., Daane, K. M., Biondi, A., Stahl, J.M., Buffington, M.L., Anfora, G., & Hoelmer, K. A. (2022). Methods for rearing the parasitoid *Ganaspis brasiliensis*, a promising biological control agent for the invasive *Drosophila suzukii*. *JoVE (Journal of Visualized Experiments)*, (184), e63898.
- Smith, S. M. (1996). Biological control with *Trichogramma*: advances, successes, and potential of their use. *Annual Review of Entomology*, 41(1), 375-406.
- Sosa-Calvo J., M. Forshage, M.L. Buffington (2024) Circumscription of the *Ganaspis brasiliensis* (Ihering, 1905) species complex (Hymenoptera, Figitidae), and the description of two new species parasitizing the spotted wing drosophila, *Drosophila suzukii* Matsumura, 1931 (Diptera, Drosophilidae). *Journal of Hymenoptera Research* 97: 441-470.
- Rodriguez-Saona, C., Cloonan, K. R., Sanchez-Pedraza, F., Zhou, Y., Giusti, M. M., & Benrey, B. (2019). Differential susceptibility of wild and cultivated blueberries to an invasive frugivorous pest. *Journal of Chemical Ecology*, 45, 286-297.
- Tait, G., Mermer, S., Stockton, D., Lee, J., Avosani, S., Abrieux, A., Anfora, G., Beers, E., Biondi, A., Burrack, H., Cha, D., Chiu, J. C., Choi, M., Cloonan, K., Crava, C. M., Daane, K. M., Dalton, D. T., Diepenbrock, L., Fanning, P., Ganjisaffar, M.G., Gut, L., Grassi, A., Hamby, K., Hoelmer, K.A., Ioriatti, C., Isaacs, R., Klick, J., Kraft, L., Loeb, G., Rossi-Stacconi, M.V., Nieri, R., Ferdinand, P., Puppato, S., Rendon, D., Renkema, J., Rodriguez-Saona, C., Rogers, M., Sassù, F., Shöneberg, T., Scott, M.J., Seagraves, M., Sial, A., Van Timmeren, S., Wallingford, A., Wang, X., Yeh, D.A., Zalom, F.G., & Walton, V. M. (2021). *Drosophila suzukii* (Diptera: Drosophilidae): A decade of research towards a sustainable integrated pest management program. *Journal of Economic Entomology*, 114(5), 1950–1974.
- Tochen, S., Dalton, D. T., Wiman, N., Hamm, C., Shearer, P. W., & Walton, V. M. (2014). Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. *Environmental Entomology*, 43(2), 501–510.
- Tonina, L., Mori, N., Sancassani, M., Dall'Ara, P., & Marini, L. (2018). Spillover of *Drosophila suzukii* between noncrop and crop areas: implications for pest management. *Agricultural and Forest Entomology*, 20(4), 575-581.
- Urbaneja-Bernat, P., Polk, D., Sanchez-Pedraza, F., Benrey, B., Salamanca, J., & Rodriguez-Saona, C. (2020). Non-crop habitats serve as a potential source of spotted-wing drosophila (Diptera: Drosophilidae) to adjacent cultivated highbush blueberries (Ericaceae). *The Canadian Entomologist*, 152(4), 474-489.
- Vinson, S. B. (1976). Host selection by insect parasitoids. *Annual review of entomology*, 21(1), 109-133.

- Vosteen, I., Van Den Meiracker, N., & Poelman, E. H. (2020). Gone with the wind: low availability of volatile information limits foraging efficiency in downwind-flying parasitoids. *Animal behaviour*, 165, 59-70.
- Waage, J. K., & Hassell, M. P. (1982). Parasitoids as biological control agents—a fundamental approach. *Parasitology*, 84(4), 241-268.
- Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V.M., O’Neal, S.D., & Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, 2(1), G1-G7.
- Wang, X. G., Nance, A. H., Jones, J. M., Hoelmer, K. A., & Daane, K. M. (2018). Aspects of the biology and reproductive strategy of two Asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii*. *Biological Control*, 121, 58-65.
- Wang, X., Kaçar, G., & Daane, K. M. (2019). Temporal dynamics of host use by *Drosophila suzukii* in California’s San Joaquin Valley: Implications for area-wide pest management. *Insects*, 10(7), 206.
- Wang, X., Hogg, B. N., Hougardy, E., Nance, A. H., & Daane, K. M. (2019). Potential competitive outcomes among three solitary larval endoparasitoids as candidate agents for classical biological control of *Drosophila suzukii*. *Biological Control*, 130, 18-26.
- Woltz, J. M., Donahue, K. M., Bruck, D. J., & Lee, J. C. (2015). Efficacy of commercially available predators, nematodes and fungal entomopathogens for augmentative control of *Drosophila suzukii*. *Journal of applied entomology*, 139(10), 759-770.

Chapter 3: Impacts of abiotic factors on overwintering survival of *Ganaspis kimorum*

Abstract

The invasive species, *Drosophila suzukii*, is a generalist pest affecting berry crops and cherries across the United States. Currently, chemical control is the main method to suppress populations in agricultural fields, but efforts are being made to implement parasitoids as biocontrol agents. In 2022, *Ganaspis kimorum* was approved for study and release in Michigan. This species uses its host's puparia to protect itself during its development, while it overwinters under leaf litter and snow cover. This provides an insulative layer to protect from lethal temperatures during the harsh winter. I performed a field study over two winter seasons to evaluate *G. kimorum*'s ability to survive winter conditions in Michigan. Location, exposure time, cumulative temperatures below freezing, and daily accumulation of snow cover, were factors that explained some of the variation in *G. kimorum* survival. This study provides evidence that up to 30% of diapausing *G. kimorum* can survive 9 weeks of exposure to the coldest period of winter in Michigan when in contact with the ground and protected under leaf litter.

Introduction

An invasive pest of thin-skinned fruit, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) originates from Southeast Asia (Kanzawa, 1939). First detected in California in 2008 and spreading to the east coast in a span of three years, it has since become a major pest in North America and temperate regions globally where blueberries, caneberries, cherries, and strawberries are grown (Lee et al., 2011, Asplen et al., 2015). Because of its disruption to existing pest management programs and devastating economic impact, there is growing interest in investigating alternative approaches to its management (Tait et al., 2021).

In Michigan, endemic parasitoid species are incapable of overcoming an immune response present in *D. suzukii*, creating a need for more effective natural enemies to regulate *D. suzukii* populations (Fleury et al., 2009, Kacsoh & Schlenke, 2012, Lee et al., 2019). Collected from its native range in Japan 2011 (Girod et al., 2018, Nomano et al., 2017) and studied under quarantine in the United States to determine host specificity and secondary interactions with native fauna (Daane et al., 2016, Girod et al., 2018, Giorgini et al., 2019), *Ganaspis kimorum* Buffington (formerly *G. brasiliensis* G1 strain) (Hymenoptera: Figitidae) (Sosa-Calvo et al. 2024) was approved for mass rearing and release as a biological control agent against *D. suzukii* (Rossi-Stacconi et al., 2022). The parasitoid prefers the 2nd instar larval stage of *D. suzukii*, laying an egg inside the larva and suppressing the immune system of the host (Wang et al., 2018). The fly larva continues to develop into a pupa, at which time the wasp larva becomes an ectoparasite, eventually consuming the fly pupa before adulthood. Development of *G. kimorum* is dependent on its host puparium for protection and maintaining appropriate conditions for pupation.

The puparia of *D. suzukii* may provide insufficient protection against extreme cold conditions during Michigan winters. Sensitive to freezing temperatures as adults and pupae, *D. suzukii* engages in a freeze avoidant strategy to reduce risk of freezing by seeking shelter (Kimura 2004, Dalton et al. 2011, Jakobs et al. 2015, Stephens et al. 2015) and overwinters as mature adult flies. Freeze avoidant strategies employed by overwintering insects help to overcome biological limitations of the environment (Izquierdo 1991, Hoffmann et al. 2002). It is thought that *D. suzukii* avoid winter air temperature extremes by overwintering under leaf litter and snowpacks, which insulate them and keep them in contact with stable ground temperatures near 0°C (Bale & Hayward, 2010). *G. kimorum* presumably has less control over where it can take shelter from winter conditions, since it relies on where the *D. suzukii* larva it infested, drops to the ground before pupating; hence the diapaused parasitoid is dependent on the protection and location of where the puparium landed when it enters diapause.

The aim of this 2-year study was to determine the overwintering potential of *G. kimorum* under field conditions and identify abiotic factors associated with survival at sites across the western Lower Peninsula of Michigan. Sites were selected from major blueberry or tart cherry production areas that have been impacted by *D. suzukii*, and along a latitudinal gradient. Abiotic factors evaluated in this study included ambient air and ground temperatures and cumulative snow cover.

Materials and methods

Site selection

To determine the overall fitness of *G. kimorum* under winter conditions in Michigan, I exposed wasp pupae to outdoor conditions from 11 January to 15 March 2023, repeating the experiment from 4 January to 29 February 2024. Four experimental locations were chosen near Benton Harbor, Hart, Suttons Bay, and East Lansing, Michigan (Figure 2.1). Locations were selected to represent the main commercial fruit production areas in the state along a north to south latitudinal gradient (Table 2.1). Each site contained cultivated blueberry or tart cherry plantings adjacent to wooded edges with wild hosts that serve as overwintering habitat for spotted-wing drosophila (Stephens et al. 2015, Wallingford et al. 2018).



Figure 3.1. Map of field sites spanning across Michigan, located in Suttons Bay, Hart, East Lansing, and Benton Harbor.

Table 3.1. Experimental sites for *G. kimorum*, GPS coordinates, and adjacent crop system.

Site	Latitude	Longitude	Crop
Suttons Bay	44°55'9.49" N	85°38'18.31" W	<i>Prunus cerasus</i>
Hart	43°42'28.40" N	86°17'58.92" W	<i>Prunus cerasus</i>
East Lansing	42°42'29.95" N	84°29'50.22" W	<i>Vaccinium corymbosum</i>
Benton Harbor	42°5'5.67" N	86°20'58.92" W	<i>Vaccinium corymbosum</i>

Wasp rearing

A colony of *G. kimorum* was initiated in March 2022 from wasps obtained from a USDA-ARS laboratory in Beltsville, MD. Wasps were reared on early instar larvae of *D. suzukii* in blueberries (Rossi-Stacconi et al., 2022). To infest the blueberries with *D. suzukii*, mature flies were obtained from a colony initiated from infested fruit collected in Michigan in 2021 and reared on standard cornmeal diet (Dalton et al., 2011) in 40-ml polystyrene drosophila culture vials with foam stoppers (Genesee Scientific, San Diego, CA). Flies were transferred to a new diet twice a week to allow for sanitation and egg laying and maintained at $25 \pm 2^\circ\text{C}$ with a 16h L:8h D light cycle and 65% relative humidity (RH). Conventionally grown blueberries were purchased from a local supermarket and soaked in DI water for 30 min, three times, to remove surface residues. Rinsed blueberries were transferred to 1 gal tubs (1.8L borosilicate glass, 11174000 OXO) and sprinkled with yeast (Fleischmann's Active Dry Yeast) to inhibit mold growth and help increase infestation by *D. suzukii*. Tubs with mesh lid covers received 30-50 flies (males and females) anesthetized with CO_2 and were maintained under the same conditions as described above for approximately 48 hours to allow for egg laying. After two days, most of the flies were released into a mesh cage, with some allowed to continue laying eggs to ensure the preferred larval stage was available to the wasps. Approximately 50 male and 50 female wasps were then added to each tub by lightly tapping on their holding vial. The wasps were given 5

days to parasitize host larvae present in the blueberries under similar environmental conditions as described above. Tubs were opened inside a mesh cage to release egg laying wasps, which were collected and incorporated back into the wasp colony. Normal colony maintenance involved incubating tubs under the same environmental conditions as described above for 3-4 weeks to facilitate adult emergence. Upon wasp emergence, approximately 50 pairs (50 females and 50 males) were aspirated into holding vials containing a roll of moistened paper towel in the bottom and a drop of honey on the underside of the foam plug. Holding vials were inverted and placed in an incubator set to the same environmental conditions described above.

Collection of puparia for cold acclimation and diapause induction

A subset of rearing tubs as described above were set aside for the experiment after egg laying wasps were released. Tubs were placed into a 14°C, 70% RH, 12:12 (L:D) h chamber to induce diapause in the larval stage of the wasps, which begins at 16°C (Hougardy et al., 2019), while allowing unparasitized *D. suzukii* to continue to develop and emerge as adults. 48h after the start of *D. suzukii* fly emergence, the tubs were then moved to a chamber at 10°C, 70% RH, 12:12 (L:D) h and held for 2 weeks. To collect puparia, each tub was placed onto an ice bath and puparia removed from blueberries, placed onto wet cotton pads in groups of 25, and then placed into a sachet bag (Hopttreely, Shenzhen City, China) which was cinched closed for the remainder of the experiment. Puparia sachets were transported to the field in an insulated ice-filled cooler to prevent exposure to warmer temperatures.

Overwintering arenas

Plastic 32 oz deli containers served as arenas for this experiment (Figure 3.2A). Upon arrival at each site, a cup cutter was used to obtain a soil core to place inside each arena. A sachet bag with parasitized puparia was placed on top of the soil core and covered with 1 inch of leaf

litter as an insulation layer. A fine fabric mesh (15 x 15 cm sheet, 0.3 mm openings) was placed over the cup and secured with a rubber band. The arena was then placed inside of the hole left behind by the cup cutter so that the sachet bag and leaf litter were at the soil level. Each hole was then covered with a wire mesh (12.7 x 12.7 cm sheet, 3 mm openings) and secured with a field staple to deter predators (Stockton et al., 2019) (Figure 3.2B).

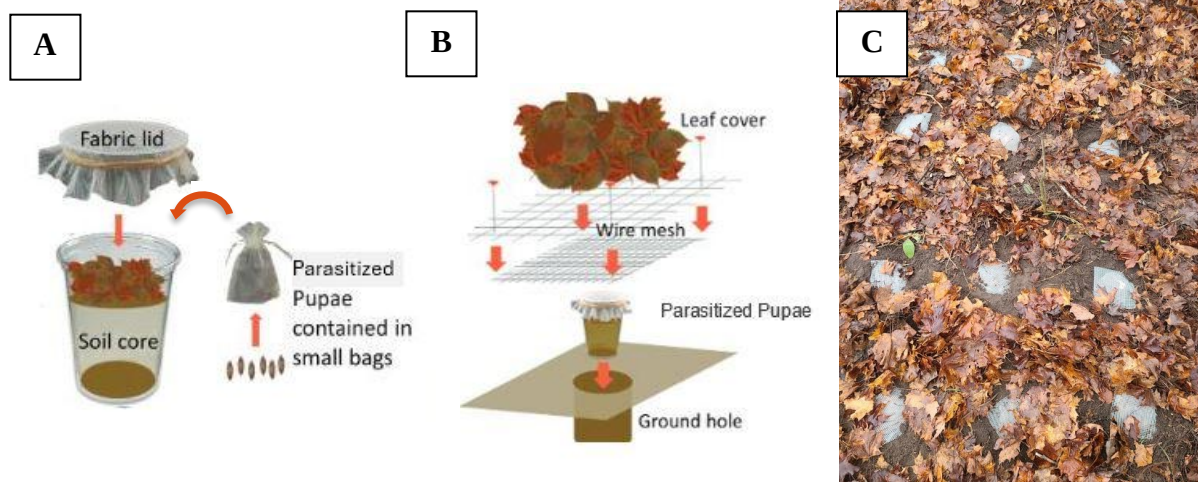


Figure 3.2. Diagram of arena assembly: parasitized pupae contained in small mesh bag, covered by leaf litter, secured by fabric lid, and placed onto soil core (A). Diagram of arenas placed into ground and covered by wire mesh and leaf litter (B). Image of arenas installed in a gridded arrangement at a field site (C). *Diagrams modified slightly from Stockton et al.*

Arena deployment and collections

Each of the four field sites were randomly assigned a set of 15 arenas, which were arranged in an array of 3x5 (Figure 3.2C) within the first 10 m of the wooded edge. Arenas were deployed to each site on 11 January 2023 and on 4 January 2024. Five arenas were collected from each site at 21, 42, and 64 days after deployment. Sachet bags were transferred to individual small plastic containers with mesh lids and several layers of saturated paper towel to prevent desiccation of puparia. Containers were placed in a mesh cage to prevent emerging wasps from escaping. Honey was provided on foam plugs as a food source for newly emerged

wasps. Cages were checked daily for 4 weeks to monitor wasp survival and emergence. Any wasps that emerged were counted as survivors. In 2023, some emerged wasps escaped the bug dorm containing bins from a given site. These wasps were counted and redistributed randomly across the 5 replicates, since they had completed development and were exposed to the same conditions from the same site and time point.

Abiotic data collection

Temperature data was recorded at each locality using HOBO data loggers (Onset Computer Corporation, Bourne, MA; Model 118b). Loggers were positioned in an identical experimental apparatus just below the leaf litter in place of a sachet. A second logger was attached to a branch under a solar shield, approximately 3 m above ground, to record ambient air temperature. Data was collected every hour for the duration of the experiment. Snow cover data was obtained using GPS coordinates for each site through Ventusky Weather Maps (<https://www.ventusky.com>).

Data analysis

Temperature recorded by HOBO loggers were used to compare the four sites and duration of exposure using two-way analysis of variance (ANOVA) and general linear hypothesis testing (GLHT) using Tukey contrasts ($\alpha = 0.05$) and multiple means comparisons in RStudio. Analysis was performed using a logistic regression (`glm(y~x, family=binomial)`) to create a model reflecting environmental factors including ambient air temperature, below ground temperature, the total number of days with below freezing conditions, and snow cover to determine their effects on survivorship. Survival was determined using the number of living and dead wasps from 3 different durations of exposure (3wks, 6wks, and 9wks) across each site. Alive and dead wasps were bound as a single factor (`cbind(alive, dead)`). First, we tested for

significant differences across timepoints using ‘emmeans’ with Tukey’s HSD to isolate time from the main model, while replicate and site were included as random effects. Then, we compared survival across the four sites and each timepoint using site and exposure time as fixed effects. Data across sites was separated by timepoint since there was a significant interaction between site and timepoint and were separated using ‘emmeans’ with Tukeys HSD. Lastly, a generalized linear mixed effect model was used to determine the impact of exposure time on survival with four covariates: daily mean ground temperatures, cumulative ground temperatures below zero (days), cumulative air temperatures below zero (days), and average daily snow fall (cm). These factors were incorporated into a model with replicate and site included as random effects, to determine their impacts on survivorship. Model of best fit was determined by the lowest delta Akaike’s information criterion (AICc) value. Multiple models were produced with a simpler subset of possible combinations of factors and compared to the full model to find the model of best fit. Model selection included these variables compared to a null model and were ranked according to their AIC values. Variance inflation factors were checked to remove multicollinearity. Akaike’s weight was used to measure uncertainty and the probability that the chosen model would be the best fit. Using R package ‘MuMIn’, the marginal and conditional R-squared values were found from the models (Bartoń & Coe, 2009). Analyses were conducted in R (version 4.4.0; R Foundation for Statistical Computing [x86_64-w64-mingw32/x64]; Vienna, Austria).

Results

No wasps emerged from arenas deployed in 2024 due to puparia handling that led to desiccation and 100% mortality, so only the 2023 field results are shown here. The highest-ranking model (Table 2.2) revealed a significant interaction among the abiotic variables of cumulative days with ground temperature below 0°C, cumulative days with air temperatures below 0°C, mean ground temperature, and mean daily snowfall accumulation on wasp survival ($P = 0.0335$). Temperatures at ground level, where arenas were buried and covered with leaf litter were more moderated and significantly different than the ambient air temperature in the 2023 winter season ($F = 76.28$; $df = 1$; $P < 0.001$) (Figure 3.3C). Ambient air temperatures fluctuated between -2.49° and 0.54° C, averaging just below freezing at -0.84° C, while ground temperatures ranged between 0.11° and 1.86 ° C, and averaging above freezing at 1.15° C (Figure 3.3C). Daily snow accumulation and cumulative days when temperatures dropped below 0°C, both air and ground temperatures, were inversely correlated with the survivorship of *G. kimorum* (Figure 3.4B). Daily snow accumulation accounted for only 3% of the variation in survivorship, while ambient air and ground temperatures accounted for a total of 26% of the variation (Table 3.3).

Site and exposure duration also explained some of the variation in survival (Figure 3.5). East Lansing, the site furthest inland from Lake Michigan, had the highest proportion of wasp emergence at 3 and 9 weeks than any other site (Figure 3.5). Survival decreased between 3 and 9 weeks of exposure at all sites (Figure 3.5). There was no significant difference between sites at the 6-week exposure timepoint, but this is also the timepoint when the fewest wasps emerged compared with either the 3 or 9-week exposure durations (Figure 3.5).

Table 3.2. Model selection parameters for determining the effect of abiotic factors on *G.**kimorum*.

Model rank	Factors in model	df	ΔAIC	Weight	R^2_m	R^2_c
NULL	~1	58	19.3	< 0.001	0.0	0.93
1	Days with ground temperature < 0 C, Mean ground temperature, Mean snowfall, Days with air temperature < 0 C	53	0.0	0.71	0.12	0.95
2	Days with ground temperature < 0 C, Mean ground temperature, Mean snowfall	54	1.9	0.27	0.08	0.94
3	Days with ground temperature < 0 C, Mean ground temperature	55	7.1	0.02	0.07	0.93
Models are ranked according to Akaike's information criterion (AICc). Only models with a ΔAIC of less than 10 are shown, excluding the null model. Model weights, marginal (R^2_m) and conditional (R^2_c) R-squared values are also shown. Degrees of freedom (df) for each model is calculated with the number of variance parameters plus the number of fixed effect coefficients.						

Table 3.3. Top ranked glmer model evaluating the effect of abiotic factors on *G. kimorum* winter survival.

Factor	R^2	SE ^a	z-score	P-Value
Days with ground temperature < 0 C	-0.26	0.02	-3.93	<0.001
Mean ground temperature	0.1	0.31	-1.15	0.25
Mean daily snowfall	-0.03	0.61	-3.40	<0.001
Days with air temperature < 0 C	0.1	0.03	2.10	0.03
^a Standard Error				

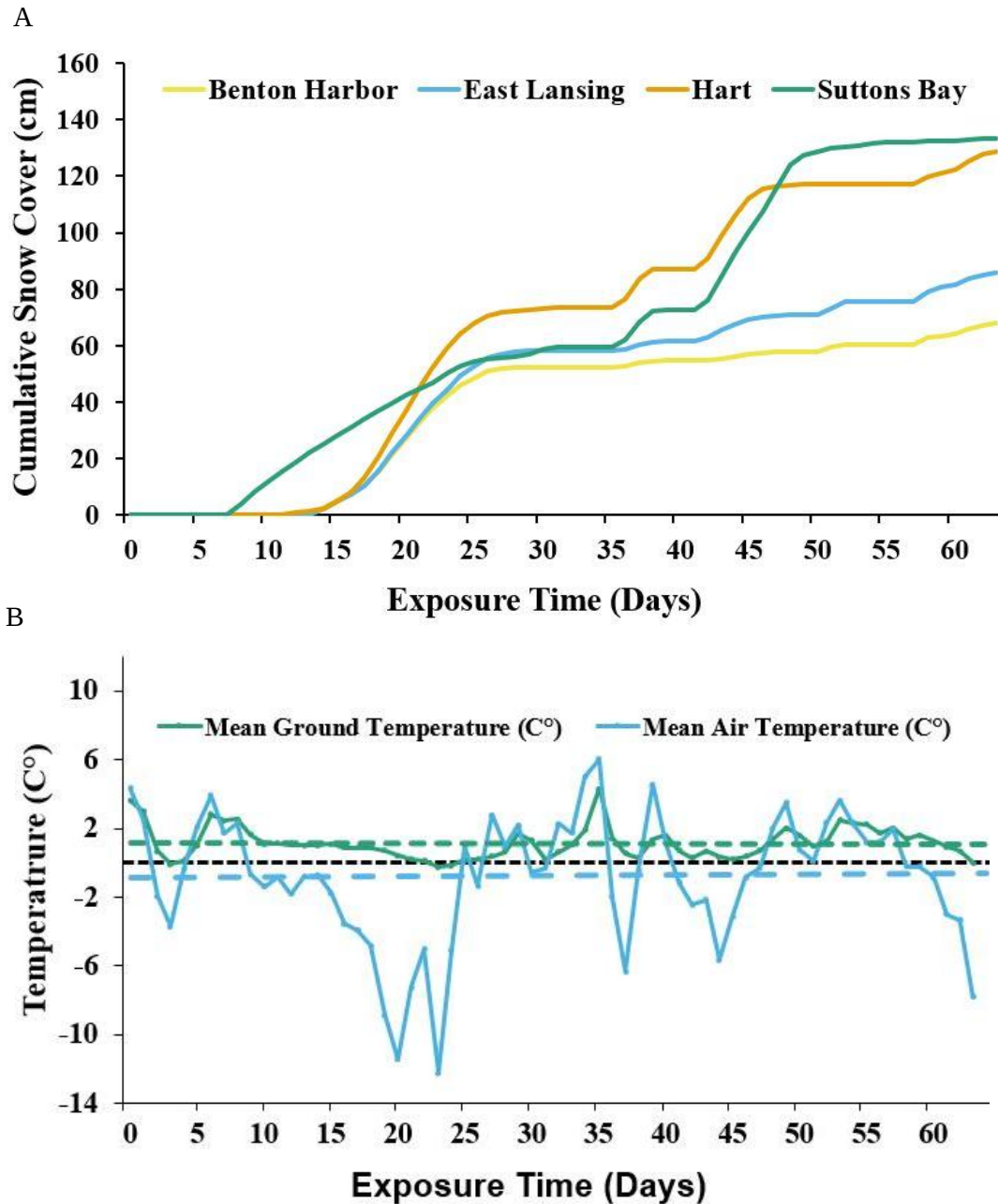
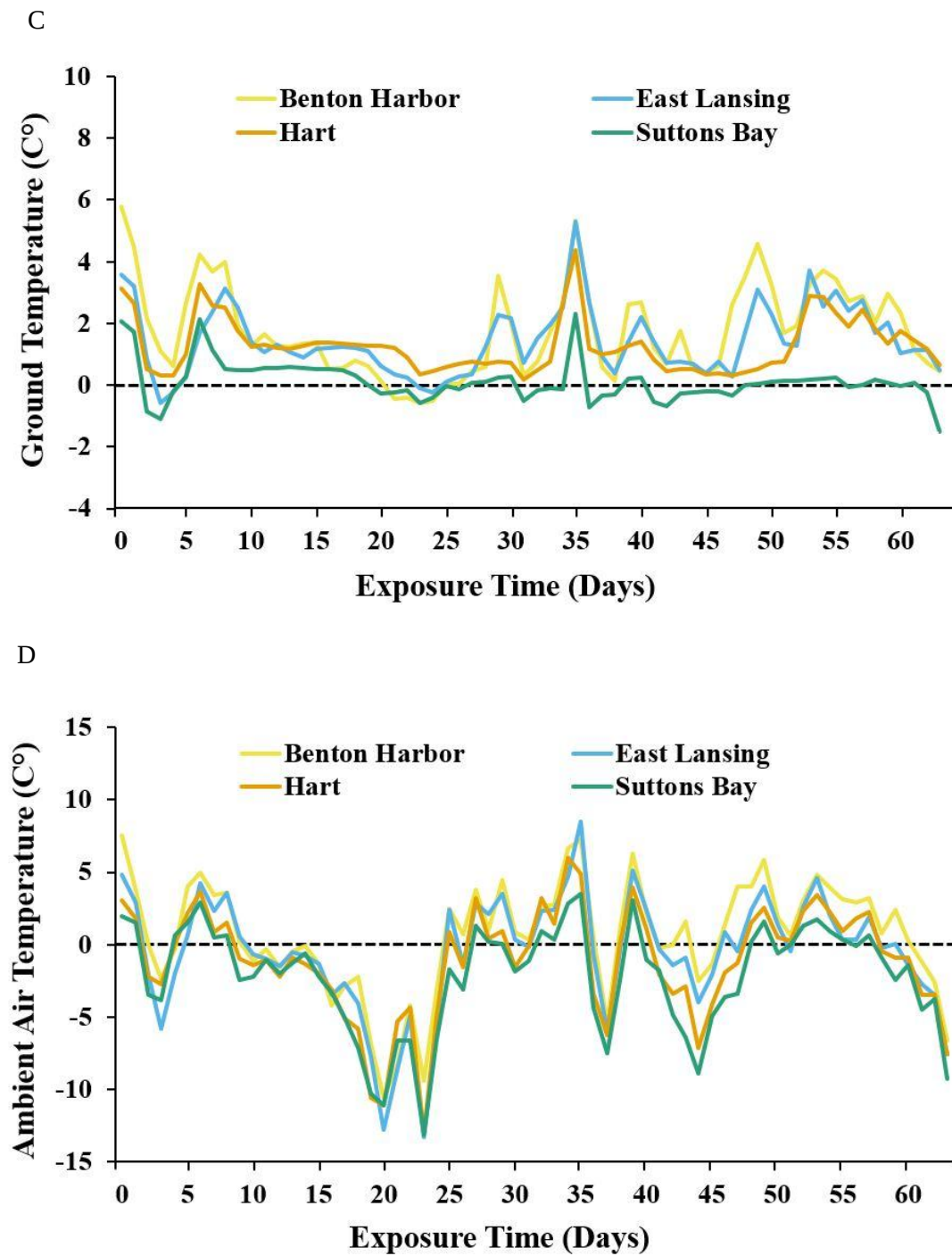


Figure 3.3. Average daily temperatures and cumulative snow fall over the course of study (64 days) in 2023. Cumulative daily snowfall at each site over the course of the experiment (A). Mean comparison of all sites' ground and air temperatures with average temperature plotted for both levels (B). Ambient temperature within the arenas (ground temperatures) at each site (C). Ambient air temperatures at each site (air temperatures) (D).

Figure 3.3. (cont'd)



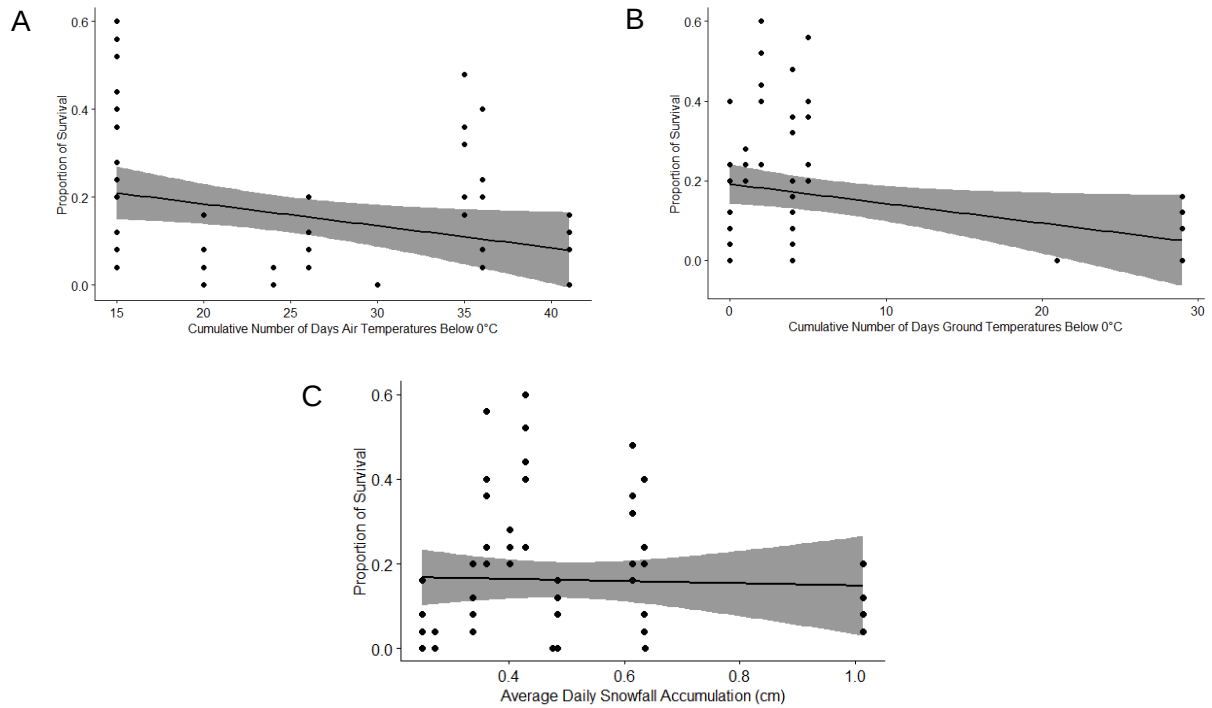


Figure 3.4. Linear regression analyses evaluating the influence of abiotic factors on survivorship in *G. kimorum*. Each graph represents a significant relationship between survivorship and an abiotic factor (R^2) using Kendall's Tau test. Cumulative days where air temperatures were below 0° C (A), cumulative days where ground temperatures were below 0° C (B), and average daily snowfall accumulation (C).

Table 3.4. Glmer model evaluating experimental site differences in *G. kimorum* survival across 3-week, 6-week, and 9-week exposure intervals.

Site Contrast	Week 3				Week 6				Week 9			
	OR	SE	Z-score	P-Value	OR	SE	Z-score	P-Value	OR	SE	Z-score	P-Value
SB*BH	0.63	0.18	-1.65	0.352	<0.01	<0.01	0.00	1.00	1.20	0.52	-1.95	0.209
SB*EL	1.45	0.38	1.42	0.4847	<0.01	<0.01	0.00	1.00	4.61	1.72	4.10	<0.001
SB*HT	0.23	0.08	-4.30	<0.001	<0.01	<0.01	0.00	1.00	2.48	0.97	2.33	0.091
HT*BH	2.74	0.96	2.87	0.021	<0.01	<0.01	0.00	1.00	0.49	0.18	-1.95	0.209
HT*EL	6.27	2.11	5.45	<0.001	<0.01	<0.01	0.00	1.00	1.86	0.56	2.05	0.169
EL*BH	0.44	0.12	-3.03	0.013	3.00	2.00	1.484	0.45	0.26	0.09	-3.10	<0.001
Key: SB = Suttons Bay, HT = Hart, EL = East Lansing, BH = Benton Harbor, OR = odds ratios, SE = standard error												

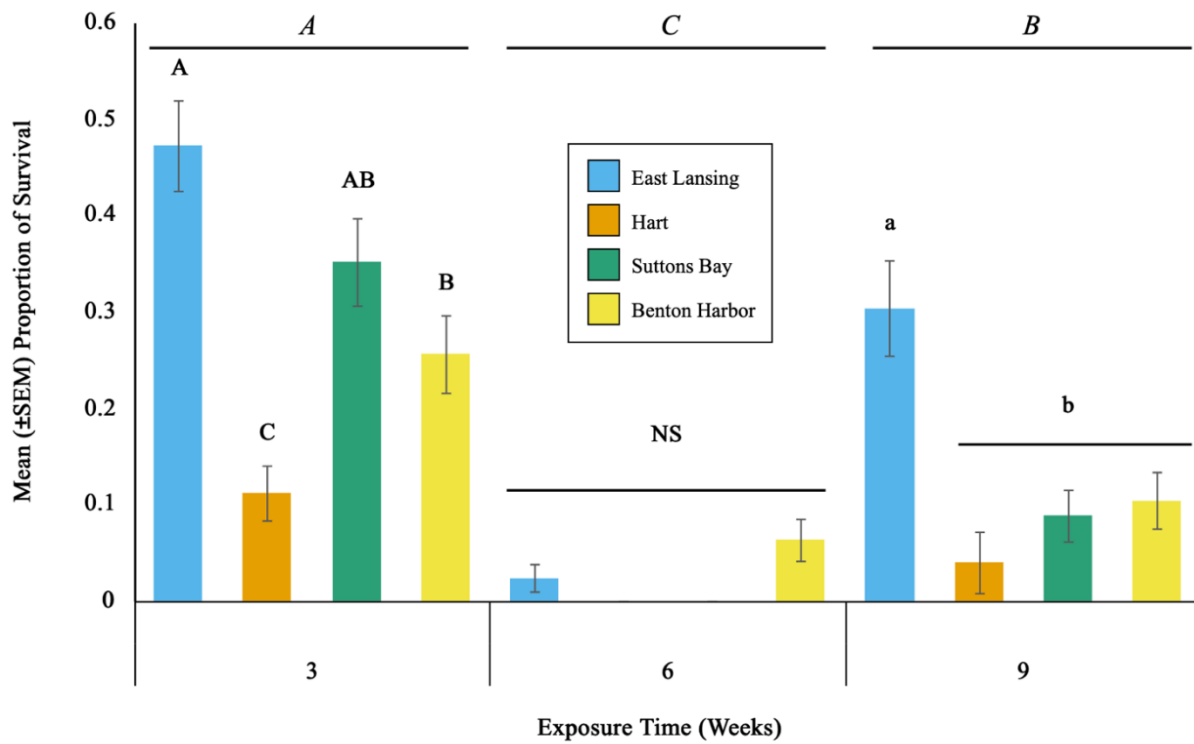


Figure 3.5. Proportion of *G. kimorum* that emerged from each site (East Lansing, Hart, Suttons Bay, Benton Harbor) and exposure duration (3, 6, 9 weeks). Means with different letters indicate statistically significant ($p < 0.05$) differences in survival between sites within each time point, and across each time point.

Discussion

This study explored the influence of winter conditions, exposure time, and location on the overwintering survival of *Ganaspis kimorum*, a novel larval parasitoid specialist of the invasive *Drosophila suzukii*, in Michigan. Although there were problems with the experiment in 2024, I was able to confirm that *G. kimorum* can survive ambient winter conditions in each of the key cherry and blueberry production areas. This finding agrees with recent studies in which two pupal parasitoids that specialize on drosophilids, *Trichopria drosophilae* and *Pachycrepoideus vindemmiae*, use a similar strategy to overwinter by pausing their development as pupae to withstand sub-zero temperatures (Amiresmaeili et al., 2020, Häner et al., 2022). We found that exposure time and location, as well as interacting abiotic factors explained some of the variability in *G. kimorum* survivorship in 2023.

One unintentional factor that likely explains lower than expected survivorship at the middle time point in 2023 and no survivorship in 2024, could be in the handling of puparia containing the diapausing wasps. In 2024, upon close examination under a microscope, it was discovered post-collection that most of the puparia had been punctured or otherwise damaged, presumably as they were transferred into the mesh bags. No wasps emerged from these puparia, and all appeared to be desiccated or were overtaken by mold, even when held under the same environmental conditions as used in 2023. Winter seasons of 2023 and 2024 had similar ambient air temperatures at -0.84°C and -0.71°C respectively, indicating that weather conditions were unlikely to be the cause of zero wasps emerging in 2024. The effect of these collection methods on our 2023 study is unknown. Of consequence, observed survivorship may be lower than expected, especially in the 6-week time point as survivorship was higher after 9-weeks of exposure to the same ambient conditions. There were two other differences between 2023 and

2024 which might also have contributed to the 2024 failure. Because of some flooding issues in 2023, drainage holes were added to the arenas in 2024 to allow for water to pass through, and perhaps this contributed to desiccation. Because some emerged wasps were able to escape into the bug dorm containing bins from a given site and time point, in 2024, sachet bags were kept separated for each replicate, rather than storing them together in a single bin by site and time point; the smaller, more confined containers may have contributed to the mold growth.

That said, we did observe abiotic effects on survival in 2023. Cumulative days with ground temperatures below freezing and snow cover were the two abiotic factors that explained some of the variability in wasp survival. It is likely that the leaf litter, snow cover, and soil into which the arenas were placed, acted as insulative protection, buffering *G. kimorum* against fluctuating air temperatures (Kimura 2004, Dalton et al. 2011, Jakobs et al. 2015, Stephens et al. 2015). Previous studies have demonstrated that insulation pockets minimize the potential for temperatures reaching lethal levels and reduce fluctuations caused by variable winter weather (Bale and Hayward 2010). It is thought that *Drosophila suzukii* uses the insulative protection of leaf litter to overwinter and persist through severe cold weather (Stockton et al., 2019), however, they do so as winter morph adults. *Ganaspis kimorum* survival depends on where *D. suzukii* larvae at the end of the growing season go to pupate, which would depend on how far from infested fruit they are likely to migrate and where the fruit they infested was growing (i.e. in the fruit crop or in wild hosts in the crop margins). Laboratory studies suggest that *G. kimorum* can survive under similar overwintering conditions as *D. suzukii*, and overwintering near its host when it emerges the following season is likely to be a co-evolved trait between host and parasitoid.

The fact that some portion of *G. kimorum* survived ambient conditions at all sites by the termination of the study in 2023 provides evidence that the parasitoid will be able to persist through the coldest part of the year in Michigan. Hougardy et al. (2019) evaluated *G. kimorum* juveniles and their intolerance of freezing temperatures and found that from 19.4°C to 17.2°C the proportion of survival dropped from approximately 90% to 0% survival and that diapause induction was important for increasing survival. East Lansing had the greatest proportion of survival from the beginning to end in comparison to our other experimental locations, which were all closer to Lake Michigan. Exposure to colder conditions may have decreased the chances of survival at sites closest to Lake Michigan, which in 2023 did not freeze over (NOAA-GLERL, <https://www.glerl.noaa.gov/data/ice/>). One variable that we did not take into account is solar radiation, which can be used as a proxy for cloud cover. East Lansing is notorious for its cloudy days, and cloud cover also acts as a buffer against fluctuating temperatures (Liepert, B.G., 2002), however, during this study, mean daily cloud cover for East Lansing was lower than the sites closer to Lake Michigan (<https://www.ventusky.com>). In this study, we artificially induced diapause based on our current understanding of this species under laboratory conditions (Hougardy et al., 2019). Under natural field conditions, diapause induction will depend on local conditions. In the future, site location is likely to be a strong determinant of *G. kimorum* survival critical to it becoming established in self-sustaining populations from year to year.

LITERATURE CITED

- Asplen, M. K., Anfora, G., Biondi, A., Choi, D. S., Chu, D., Daane, K. M., Gilbert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Véték, G., Vogt, H., Walton, V.M., Yu, Y., Zappalá, L., & Desneux, N. (2015). Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*, 88, 469-494.
- Amiresmaeili, N., Romeis, J., & Collatz, J. (2020). Cold tolerance of the drosophila pupal parasitoid *Trichopria drosophilae*. *Journal of Insect Physiology*, 125, 104087.
- Bale, J. S., & Hayward, S. A. L. (2010). Insect overwintering in a changing climate. *Journal of Experimental Biology*, 213(6), 980-994.
- Bartón, N. H., & Coe, J. (2009). On the application of statistical physics to evolutionary biology. *Journal of Theoretical Biology*, 259(2), 317-324.
- Daane, K. M., Wang, X. G., Biondi, A., Miller, B., Miller, J. C., Riedl, H., Shearer, P.W., Guerrieri, E., Giorgini, M., Buffington, M., Achterberg, K., Song, Y., Kang, T., Yi, H., Jung, C., Lee, D.W., Chung, B., Hoelmer, K.A., & Walton, V. M. (2016). First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents. *Journal of Pest Science*, 89, 823-835.
- Dalton, D. T., Walton, V. M., Shearer, P. W., Walsh, D. B., Caprile, J., & Isaacs, R. (2011). Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. *Pest Management Science*, 67(11), 1368-1374.
- Fleury, F., Gibert, P., Ris, N., & Allemand, R. (2009). Ecology and life history evolution of frugivorous *Drosophila* parasitoids. *Advances in Parasitology*, 70, 3-44.
- Giorgini, M., Wang, X. G., Wang, Y., Chen, F. S., Hougardy, E., Zhang, H. M., Chen, Z.Q., Chen, H.Y., Liu, C.X., Cascone, P., Formisano, G., Carvalho, G.A., Biondi, A., Buffington, M., Daane, K.M., Hoelmer, K.A., & Guerrieri, E. (2019). Exploration for native parasitoids of *Drosophila suzukii* in China reveals a diversity of parasitoid species and narrow host range of the dominant parasitoid. *Journal of Pest Science*, 92, 509-522.
- Girod, P., Lierhmann, O., Urvois, T., Turlings, T. C., Kenis, M., & Haye, T. (2018). Host specificity of Asian parasitoids for potential classical biological control of *Drosophila suzukii*. *Journal of Pest Science*, 91, 1241-1250.
- Häner, N., Amiresmaeili, N., Stähli, N., Romeis, J., & Collatz, J. (2022). Overwintering of two pupal parasitoids of *Drosophila* under natural conditions. *Journal of Thermal Biology*, 106, 103231.

- Hoffmann, A. A., Anderson, A., & Hallas, R. (2002). Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecology letters*, 5(5), 614-618.
- Hougardy, E., Hogg, B. N., Wang, X., & Daane, K. M. (2019). Comparison of thermal performances of two Asian larval parasitoids of *Drosophila suzukii*. *Biological control*, 136, 104000.
- Izquierdo, J. I. (1991). How does *Drosophila melanogaster* overwinter?. *Entomologia Experimentalis et Applicata*, 59(1), 51-58.
- Jakobs, R., Garipey, T. D., & Sinclair, B. J. (2015). Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. *Journal of Insect Physiology*, 79, 1-9.
- Kacsoh, B. Z., & Schlenke, T. A. (2012). High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. *PloS one*, 7(4), e34721.
- Kanzawa, T. (1939). Studies on *Drosophila suzukii* Mats. Kofu, Yamanashi Agricultural Experiment Station, 49 pp. *Review of Applied Entomology*, 29, 622 (abst.).
- Kimura, M. T. (2004). Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia*, 140, 442-449.
- Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Van Steenwyk, R. A., & Yorgey, B. M. (2011). The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Management Science*, 67(11), 1358-1367.
- Lee, J. C., Wang, X., Daane, K. M., Hoelmer, K. A., Isaacs, R., Sial, A. A., & Walton, V. M. (2019). Biological control of Spotted-Wing Drosophila (Diptera: Drosophilidae) current and pending tactics. *Journal of Integrated Pest Management*, 10(1).
- Liepert, B. G. (2002). Observed reductions of surface solar radiation at sites in the United States and worldwide from 1961 to 1990. *Geophysical research letters*, 29(10), 61-1.
- Nomano, F. Y., Kasuya, N., Matsuura, A., Suwito, A., Mitsui, H., Buffington, M. L., & Kimura, M. T. (2017). Genetic differentiation of *Ganaspis brasiliensis* (Hymenoptera: figitidae) from East and Southeast Asia. *Applied Entomology and Zoology*, 52, 429-437.
- Rossi-Stacconi, M. V., Wang, X., Stout, A., Fellin, L., Daane, K. M., Biondi, A., Stahl, J.M., Buffington, M.L., Anfora, G., & Hoelmer, K. A. (2022). Methods for rearing the parasitoid *Ganaspis brasiliensis*, a promising biological control agent for the invasive *Drosophila suzukii*. *JoVE (Journal of Visualized Experiments)*, (184), e63898.
- Sosa-Calvo, J., Forshage, M., & Buffington, M. L. (2024). Circumscription of the *Ganaspis brasiliensis* (Ihering, 1905) species complex (Hymenoptera, Figitidae), and the

- description of two new species parasitizing the spotted wing drosophila, *Drosophila suzukii* Matsumura, 1931 (Diptera, Drosophilidae). *Journal of Hymenoptera Research*, 97, 441-470.
- Stephens, A. R., Asplen, M. K., Hutchison, W. D., & Venette, R. C. (2015). Cold hardiness of winter-acclimated *Drosophila suzukii* (Diptera: Drosophilidae) adults. *Environmental Entomology*, 44(6), 1619-1626.
- Stockton, D., Wallingford, A., Rendon, D., Fanning, P., Green, C. K., Diepenbrock, L., Ballman, E., Walton, V.M., Isaacs, R., Leach, H., Sial, A.A., Drummond, F., Burrack, H., & Loeb, G. M. (2019). Interactions between biotic and abiotic factors affect survival in overwintering *Drosophila suzukii* (Diptera: Drosophilidae). *Environmental Entomology*, 48(2), 454-464.
- Tait, G., Mermer, S., Stockton, D., Lee, J., Avosani, S., Abrieux, A., Anfora, G., Beers, E., Biondi, A., Burrack, H., Cha, D., Chiu, J. C., Choi, M., Cloonan, K., Crava, C. M., Daane, K. M., Dalton, D. T., Diepenbrock, L., Fanning, P., Ganjisaffar, M.G., Gut, L., Grassi, A., Hamby, K., Hoelmer, K.A., Ioriatti, C., Isaacs, R., Klick, J., Kraft, L., Loeb, G., Rossi-Stacconi, M.V., Nieri, R., Ferdinand, P., Puppato, S., Rendon, D., Renkema, J., Rodriguez-Saona, C., Rogers, M., Sassù, F., Shöneberg, T., Scott, M.J., Seagraves, M., Sial, A., Van Timmeren, S., Wallingford, A., Wang, X., Yeh, D.A., Zalom, F.G., & Walton, V. M. (2021). *Drosophila suzukii* (Diptera: Drosophilidae): A decade of research towards a sustainable integrated pest management program. *Journal of Economic Entomology*, 114(5), 1950–1974.
- Wallingford, A. K., Rice, K. B., Leskey, T. C., & Loeb, G. M. (2018). Overwintering behavior of *Drosophila suzukii*, and potential springtime diets for egg maturation. *Environmental Entomology*, 47(5), 1266-1273.
- Wang, X. G., Nance, A. H., Jones, J. M., Hoelmer, K. A., & Daane, K. M. (2018). Aspects of the biology and reproductive strategy of two Asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii*. *Biological Control*, 121, 58-65.

Chapter 4: Conclusions and future directions

This thesis provides valuable insights into the work being done to establish *G. kimorum*, a novel parasitoid, as a successful biocontrol agent against *D. suzukii*. Methods for control of *D. suzukii* have relied on chemical control and lack impact on significant populations of the pest in wild areas. Biocontrol, specifically parasitoids, offers a self-sustaining population of host-specific natural enemies that are able to disperse large distances to pursue their host. My thesis focuses on *G. kimorum* which was permitted for rearing and release in 2022 but has highlights on the adventive populations of *L. japonica* found through surveys of *G. kimorum* in the field. Overall, this thesis attempts to observe foundational behaviors of *G. kimorum* in Michigan and evaluate its survival and establishment success.

In chapter 2, my study centered around *G. kimorum*'s dispersal pattern after releasing them into commercial blueberry and tart cherry fields and their success in foraging for hosts. *Drosophila suzukii* utilize wild fruit present in unmanaged areas adjacent to the crop to increase populations in the early season, then invade when the crop is ripe prior to harvest. Current management practices can reduce invasion but do not directly affect pest populations in these unmanaged areas. It is unknown if *G. kimorum* can provide the control needed to reduce *D. suzukii* populations or successfully forage for their hosts in the field after release. To observe dispersal patterns of *G. kimorum*, preliminary surveys of release sites were conducted to confirm no existing populations existed, observe potential competition of other parasitoids, and ensure hosts were available. Using baited fruit in sentinel traps, collecting wild and cultivated fruit, and placing adult *D. suzukii* traps, records of impactful insect populations were recorded. At the time of release, sentinel traps were placed in a T-shaped pattern with two transects parallel to the treeline and one transect intersecting with the crop. Three sentinel traps were placed at each

intersection at various distances to measure distance and direction that the wasps traveled after release. After releases, sentinel trapping and fruit collections continued to evaluate if *G. kimorum* had successfully produced additional generations. All collected traps and fruit were reared under colony conditions, and emerged parasitoids were recorded. Results showed that no *G. kimorum* emerged from any sentinel traps and was not detected at any of our cherry orchards. Tart cherry had low fruit availability and low *D. suzukii* population density, which would decrease suitability for *G. kimorum* and likely forced the parasitoids to seek a more suitable habitat. This is supported by the low number of other parasitoids that emerged from sentinel traps compared to blueberry sites. In blueberry, fruit collection detected *G. kimorum* at multiple sites indicating that the parasitoids successfully found hosts and produced a second generation. However, a large proportion of *G. kimorum* detected was at one site, reducing confidence in its establishment success in other regions. Low disturbance management strategies promote natural growth of non-crop hosts and biodiversity, increasing refuge and hosts for *G. kimorum* and *L. japonica*. Fruit collections and sentinel traps rely on fully developed parasitoids to exit fruit, leaving an unknown quantity of parasitized hosts unaccounted for. This indicates that our sampling methods, especially sentinel trapping, do not accurately describe *G. kimorum* populations or represent total abundance of parasitoids. Of note, there was a large proportion of *L. japonica* in both sampling methods in blueberry fields. *Leptopilina japonica* is an adventive generalist parasitoid of drosophilids, first detected in Michigan in 2022, it has had aggressive population growth and spread throughout Michigan. Its wider host range, faster development time, and lack of discrimination against previously parasitized larvae, lends itself to be a detrimental competitor to *G. kimorum*.

In chapter 3, I evaluated the overwintering strategy of *G. kimorum* by subjecting diapausing pupae to winter field conditions and reviewing abiotic factor impacts on survival. The parasitoid's strategy of freeze-avoidance is utilized by suspending its development inside of *D. suzukii* puparia during the winter months, presumably hidden under the insulative layers of snow cover and leaf litter at ground level. This approach allows it to exploit its host's behavior to seek insulation pockets that buffer variable weather conditions, while maintaining close vicinity to its host later in the season. The study exposed parasitized pupae to three regions across West Michigan's lower peninsula and one central region. This was done in 2023 and 2024, however there was no wasp emergence in 2024. This included a control set of puparia maintained in the lab under normal rearing conditions, which upon further observation of puparia it was discovered that they were punctured during collection, prior to inducing diapause. As a consequence, these methods may have negatively impacted our 2023 study, however there was a significant amount of survival at our first and last collection to run analysis on the effects of abiotic factors. In 2023, all regions had wasps emerge from the final time point after 9 weeks of exposure to winter conditions. Results showed that the highest proportion of wasps survived at our central location throughout the experiment. Of the coastal sites, our midpoint location had the highest proportion of survival, compared to the most northern and southern sites. Abiotic factors that were tested for impact included, average daily ground temperature, cumulative days ground temperatures were below freezing, cumulative days when ambient air temperatures were below freezing, and daily snowfall accumulation. Of these factors, cumulative days when ground temperatures were below zero had the highest impact on survival. Though, snow cover and cumulative days when ambient air temperatures were below freezing were significantly affecting survivorship. Overall, this study shows that *G. kimorum* can persist through the winter season across Michigan, while

indicating regions more suitable for establishment success. This study is one of the first to support that *G. kimorum* can survive under leaf litter and snow cover, which is a freeze-avoidance strategy used by the adult winter morph of its host. It also highlights abiotic factors that influence wasp survivorship and can help predict population density later in the season.

My thesis illustrates the obstacles and successes of implementing *G. kimorum* as a biocontrol agent against *D. suzukii*. My results show that *G. kimorum* are able to survive conditions similar to its host to persist through Michigan's irregular winter weather, though, overwintering success is reliant on regional weather differences. This finding can dissuade from attempts at establishing the parasitoid as a classical control at these more extreme locations and may need to be implemented as an augmentative control method with annual releases to supplement surviving populations. However, errors in collecting parasitized pupae may have contributed to low survivorship and further studies should be done to accurately measure survivorship. It is unknown if adult *G. kimorum* can overwinter and may supply more evidence to their success at sustaining populations through the winter season. My results show that *G. kimorum* is able to forage for and parasitize hosts in commercial blueberry fields and produce additional generations. While it is important to note that their populations were low, our methods for detection are in the early stages of development and may not reflect the actual population densities post-release. Future research should focus on monitoring adult *G. kimorum* populations, to accurately report on their abundance post-release. Additional studies should be done on *L. japonica* and its interactions with *G. kimorum* in the field, highlighting potential competition between these two species. Overall, this information is valuable in guiding further research and establishment efforts on *G. kimorum*, and its implementation into current IPM programs as a potential biocontrol agent in controlling *D. suzukii*.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2024-02

Author and Title of thesis:

Author: Andrew J. Jones

Title: DETERMINING THE ESTABLISHMENT POTENTIAL OF *GANASPIS KIMORUM* IN MICHIGAN

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Drosophilidae	<i>Drosophila suzukii</i>	adult	10	pinned
Figitidae	<i>Leptopilina japonica</i>	adult	10	pinned
Figitidae	<i>Ganaspis kimorum</i>	adult	10	pinned