The effects of allelochemical transfer on the dynamics of hosts, parasitoids, and competing hyperparasitoids

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Abstract
Allelochemicals produced by plants may be ingested by herbivorous insects and transferred to higher trophic levels with potentially deleterious effects. We develop a system of differential equations to investigate the effect of the transfer of allelochemicals, such as nicotine, on the population dynamics of a system of hosts, parasitoids, and two competing hyperparasitoids that attack different life stages of the parasitoids. We find both somewhat deleterious effects of nicotine on the larvae-attacking hyperparasitoids and increased attack rates for the pupae-attacking hyperparasitoids can promote coexistence. We also use an evolutionary game-theoretic approach to determine the optimal distribution of hyperparasitoid attacks among nicotine-producing and nicotine-free plants. With strong deleterious effects of nicotine and increased attack rates for the pupae-attacking hyperparasitoid, we find both species attack parasitoids on the nicotine-free plant but only pupae-attacking hyperparasitoids attack parasitoids on the nicotine-producing plant.
Recommendations for Resource Managers:

- In the absence of nicotine and with equal attack rates, the larvae-attacking hyperparasitoids will exclude pupae-attacking hyperparasitoids due to the advantage of attacking an earlier life-stage of the parasitoid.
- Coexistence of larvae- and pupae-attacking hyperparasitoid species can occur when the pupae-attacking species has the advantage of a higher oviposition rate and/or when the larvae-attacking species suffers increased mortality due to nicotine.
- If mortality rates of larvae-attacking hyperparasitoids due to nicotine become too large, they are unable to persist.
- The population dynamics of systems incorporating multiple trophic levels are complex and intertwined. The presence of allelochemicals such as nicotine in the first trophic level may impact the observed diversity of hyperparasitoid species in the fourth trophic level.

**KEYWORDS**
coexistence, Darwinian dynamics, habitat selection, hyperparasitism, nicotine

1 | INTRODUCTION

The population dynamics of systems of interacting herbivorous hosts, parasitoids, and hyperparasitoids are affected by a lower trophic level—the host plants on which the herbivorous host feeds. Some plants produce allelochemicals as a defense mechanism against herbivory, and in turn, some herbivorous insects have evolved behavioral and physiological adaptations to overcome and specialize on plants that produce these specific allelochemicals. Plant allelochemicals are then transferred from specialist hosts to parasitoids and hyperparasitoids, with potentially deleterious effects.

Endophagous parasitoids deposit eggs and complete larval development within their herbivorous hosts, resulting in the eventual death of their hosts (Godfray et al., 1994). These primary parasitoids are themselves vulnerable to higher trophic-level parasitoids known as “hyperparasitoids,” or “secondary parasitoids” (Sullivan, 1987). Both primary and secondary parasitoids vary in their ovipositional strategies; for example, some species attack the larval stage of the host or primary parasitoid, whereas others attack the prepupal or pupal stages (Godfray et al., 1994).
The tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), is a specialist on the Solanaceae, which includes many alkaloid-producing species (Chowański et al., 2016). In North America, it is a common pest of cultivated tobacco (*Nicotiana tabacum* L.), which produces nicotine, and tomato (*Lycopersicon esculentum* Mill.), which does not produce nicotine. *Manduca sexta* is highly tolerant to alkaloids and particularly insensitive to nicotine, due to the rapid and efficient degradation of nicotine by inducible enzymes and excretion of nicotine metabolites (Self et al., 1964a, 1964b; Snyder et al., 1994; Wink & Theile, 2002). Although most of the ingested nicotine is metabolized and excreted, enough remains in the host hemolymph to affect parasitoids and hyperparasitoids (Barbosa et al., 1986; Harvey et al., 2007).

*Cotesia congregata* (Say) (Hymenoptera: Braconidae) is the only hymenopterous endoparasitoid that attacks larvae of *M. sexta*. This parasitoid is gregarious, that is, females deposit multiple eggs into a host during a single ovipositional event; the preferred host stage is the third of five instars. Parasitoid larvae feed on nutrients dissolved in the host hemolymph and are thus exposed to low levels of undegraded nicotine that can deleteriously affect their development and survival (Barbosa et al., 1986). However, populations of *C. congregata* associated historically with *M. sexta* on tobacco have evolved behavioral and physiological adaptations, resulting in greater tolerance to nicotine (Kester & Barbosa, 1994).

While undergoing development in *M. sexta*, *C. congregata* is vulnerable to parasitism by several hymenopterous hyperparasitoids, including species that attack larvae, such as *Mesochorus americanus* Cresson (Ichenumonidae), and several species, including *Hypopteromalus tabacum* (Fitch) (Pteromalidae), that attack prepupae within cocoons (McNeil & Rabb, 1973). Allelochemicals ingested by the herbivorous host can be transferred not only to the third trophic level (parasitoids) but also, subsequently, to the fourth trophic level (hyperparasitoids) (Harvey et al., 2007). Since most of the alkaloids present in *C. congregata* larvae are excreted in the meconium or shed into cocoon silk (Barbosa et al., 1986), it is likely that hyperparasitoid species that attack larval parasitoids are exposed to higher levels of nicotine than hyperparasitoids that attack prepupal or pupal parasitoids. Interestingly, the diversity of hyperparasitoid species has been observed to be lower on nicotine-producing tobacco plants compared to nicotine-free tomato plants; no larva-attacking species of hyperparasitoid have been observed emerging from *C. congregata* cocoons on tobacco plants (Karen Kester, Personal Communication).

Many mathematical models have been created and analyzed for host-parasitoid systems (Hassell, 2000; Ives, 1992; Mills & Getz, 1996; Nicholson & Bailey, 1935), including systems with multiple parasitoids attacking a single host (Comins, 1996; Hassell & May, 1986; Hogarth & May, 1984; Kakehashi et al., 1984; May & Hassell, 1981). Bonsall et al. (2002) studied a stage-structured system with a single host and two competing parasitoids, where juveniles of each parasitoid species are vulnerable to attack by adults of the other parasitoid species. Briggs (1993) explored the result of competition between two species of parasitoids attacking different life stages of the host, egg and larval. Other models have incorporated a third trophic level (Beddington & Hammond, 1977; Comins, 1996; Holt & Hochberg, 1998; May & Hassell, 1981), showing the impact of the hyperparasitoid on the host and parasitoid dynamics. The effects of hyperparasitoids can have important implications for biological control, since parasitoids are frequently used as a means of pest suppression (Tougeron & Tena, 2019).

Motivated by interactions among *M. sexta*, *C. congregata*, *M. americanus*, and *H. tabacum*, we develop a system of differential equations to investigate the effect of allelochemical transfer on the population dynamics of a system of hosts, parasitoids, and both
larval and prepupal or pupal hyperparasitoids. Our continuous-time model incorporates stage-structure for the hosts, parasitoids, and two species of hyperparasitoids within a habitat of a single plant, which may be either a nicotine-producing plant, such as tobacco, or a nicotine-free plant, such as tomato. Nicotine is assumed to affect the survival of the hyperparasitoid *M. americanus*, which attacks the larval stage of the primary parasitoid, *C. congregata*, but not the survival of *H. tabacum*, which attacks the prepupal stage of the primary parasitoid. We analyze the stability of the model’s equilibria and determine invasion criteria for the competing hyperparasitoid species. We explore how increased levels of nicotine in the system, as well as differences in oviposition rates between the two hyperparasitoid species, affect the outcome of competition between the hyperparasitoids, and discuss the implications for hyperparasitoid diversity. We focus on mechanisms that promote coexistence of the two hyperparasitoid species.

We next extend our model to a more realistic two-plant system to investigate how plant-specific oviposition rates of competing larvae-attacking and pupae-attacking hyperparasitoid species might evolve when able to attack parasitoids on both nicotine-free plants and nicotine-producing plants. We use an evolutionary game theoretic approach to determine optimal oviposition strategies, as well as the resulting equilibrium spatial distributions, for each hyperparasitoid species. We explore how results change as the effects of nicotine on the nicotine-producing plant increase, and again discuss the implications for hyperparasitoid diversity.

2 | MODEL DEVELOPMENT

We construct an ordinary differential equation model to describe the dynamics of a system of hosts, parasitoids, and two competing hyperparasitoids on a single plant species. We use this model to explore the population dynamics under varying levels of plant nicotine production.

2.1 | Host-only model

We first consider a differential equation model for the host, *M. sexta*, in the absence of parasitoids and hyperparasitoids. The life-cycle of *M. sexta* has four stages: egg, larva, pupa, and adult. All stages are modeled except for eggs. Eggs are laid by adults and hatch into larvae at rate $b$ multiplied by the density limiting factor $(1 - L/K)$. Larvae mature into pupae at rate $g_L$ and have natural mortality rate $\mu_L$. All pupae mature into adults at rate $g_P$. Adults have natural mortality rate $\mu_A$.

The host-only model equations are as follows:

$$\frac{dL}{dt} = b \left(1 - \frac{L}{K}\right)A - \mu_L L - g_L L$$

$$\frac{dP}{dt} = g_L L - g_P P$$

$$\frac{dA}{dt} = g_P P - \mu_A A$$

Model (1) has two equilibria: the extinction equilibrium, $(0, 0, 0)$, and a nonextinction equilibrium, $\left(L^*, P^*, A^*\right)$, where $L^* = \frac{K \left(b_L - \mu_L \mu_A - \mu_A L_c\right)}{b_L}$, $P^* = \frac{K \left(b_L - \mu_L \mu_A - \mu_A L_c\right)}{b_P}$, and
\[ A^* = \frac{K(b_L - \mu_A \mu_L - \mu_g g_L)}{b_{gA}}. \]

Since all model parameters are positive, in order for all components of the nonextinction equilibrium to be positive we must have \( b_L - \mu_A \mu_L - \mu_A g_L > 0 \), or equivalently,

\[
\left( \frac{b}{\mu_A} \right) \left( \frac{g_L}{\mu_L + g_L} \right) > 1. \quad (2)
\]

To determine conditions for the stability of each equilibrium we look at the eigenvalues of the Jacobian matrix evaluated at that equilibrium point. The characteristic equation for the Jacobian matrix evaluated at the extinction equilibrium has the form:

\[
a_3 \lambda^3 + a_2 \lambda^2 + a_1 \lambda + a_0 = 0, \quad (3)
\]

where \( a_3 = 1, \ a_2 = (\mu_A + g_P + \mu_L + g_L), \ a_1 = (g_L + \mu_A \mu_L + g_L g_P + \mu_A (\mu_L + g_L)), \) and \( a_0 = (\mu_L + g_L) \mu_A - g_L b g_P. \) Under the Routh-Hurwitz criteria for stability, all coefficients must be the same sign. If any coefficient’s sign differs, then there exist eigenvalues of opposite signs guaranteeing at least one eigenvalue with positive real part and an unstable equilibrium. Since all parameters are positive, it is clear that \( a_3 > 0, a_2 > 0, \) and \( a_1 > 0. \) If the criterion (2) holds, so the nonextinction equilibrium is positive, then \( a_0 < 0, \) and by the Routh-Hurwitz criteria, the extinction equilibrium will be unstable.

If instead

\[
\left( \frac{b}{\mu_A} \right) \left( \frac{g_L}{\mu_L + g_L} \right) < 1, \quad (4)
\]

then all coefficients of the characteristic equation are positive. The additional Routh-Hurwitz criteria needed for stability is \( a_1 a_2 > a_0, \) and it is easy to show this condition holds since all parameters are positive. Therefore the extinction equilibrium is stable when (4) holds and unstable when condition (2) holds.

The characteristic equation for the Jacobian matrix evaluated at the nonextinction equilibrium is of the same form as (3) with \( a_3 = 1, \ a_2 = \frac{b g_L + g_P \mu_A + \mu_A}{\mu_A}, \ a_1 = \frac{b g_L + b g_P \mu_A + g_P \mu_A^2}{\mu_A}, \) and \( a_0 = g_P (b g_L - \mu_A g_L - \mu_A \mu_P). \) If condition (2) is met, then all coefficients are positive. The nonextinction equilibrium point is stable if \( a_1 a_2 > a_0, \) which reduces to

\[
\frac{(g_P^2 + (g_L + \mu_L) g_P + b g_L) \mu_A^3 + (b g_L + b g_P g_P) \mu_A^2 + (b^2 g_L^2 + b g_L g_P + b g_L g_P^2) \mu_A + b^2 g_L^2 g_P}{\mu_A^2} > 0.
\]

This condition is satisfied since all parameters are positive. Thus, the nonextinction equilibrium in the host-only model is stable for all parameters sets for which (2) holds.

### 2.2 Host-parasitoid model

In this section we extend the model to include parasitoids, modeling four life-stages: eggs (\( P_E \)), larvae (\( P_L \)), prepupae/pupae (\( P_P \)), and adults (\( P_A \)). We note that while we will refer to \( P_P \) as the pupal class of the parasitoid, it includes both the prepupal and pupal stages. The density of parasitoid eggs, \( P_E \), increases as eggs are successfully oviposited into host larvae. We let \( c \) be the
clutch size, and $\alpha$ be the rate of successful oviposition of eggs by parasitoid adults ($P_A$) into host larvae. All parasitoid eggs ($P_E$) are assumed to mature into parasitoid larvae ($P_L$) at rate $g_{PE}$. Parasitoid larvae mature into the pupal class ($P_P$) at rate $g_{PL}$ and die at rate $\mu_{PL}$. Parasitoid pupae mature into adults at rate $g_{PP}$ and die at rate $\mu_{PP}$. Parasitoid adults have a natural mortality rate of $\mu_{PA}$.

When model (1) is coupled with the parasitoid equations we obtain the host-parasitoid model equations as follows:

\[
\begin{align*}
\frac{dL}{dt} &= b\left(1 - \frac{L}{K}\right)A - \mu_L L - g_{GL} L - \alpha P_A L \\
\frac{dP}{dt} &= g_{GL} L - g_{PP} P \\
\frac{dA}{dt} &= g_{PP} P - \mu_A A \\
\frac{dP_E}{dt} &= c\alpha P_A L - g_{PE} P_E \\
\frac{dP_L}{dt} &= g_{PE} P_E - g_{PL} P_L - \mu_{PL} P_L \\
\frac{dP_P}{dt} &= g_{PL} P_L - g_{PP} P_P - \mu_{PP} P_P \\
\frac{dP_A}{dt} &= g_{PP} P_P - \mu_{PA} P_A.
\end{align*}
\]  

Model (5) yields three equilibria:

Extinction:

\[ (0, 0, 0, 0, 0, 0, 0) \]  

Host only:

\[ (L^*, P^*, A^*, 0, 0, 0) \]  

Host and parasitoid:

\[ (L^*, P^*, A^*, P_{E*}, P_{L*}, P_{P*}, P_{A*}) \]  

Note that here and in the following sections, for convenience, we use the same notation (i.e., $L^*$, $P^*$, $A^*$, etc.) for each of the equilibria even though $L^*$ for one equilibrium in general is not necessarily equal to $L^*$ of another equilibrium.

The extinction equilibrium (6) is stable exactly when the extinction equilibrium of the host-only model is stable. Parasitoids cannot persist in the absence of the host and must also go extinct.

The second equilibrium is the host-only equilibrium (7), where $L^*$, $P^*$, and $A^*$ are identical to the positive equilibrium values for the host-only model and densities of all stages of the parasitoid population are zero. When evaluated at equilibrium (7), the Jacobian matrix of model (5) has a block structure where the upper $3 \times 3$ block is the same as the Jacobian for model (1) evaluated at the nonextinction equilibrium. All eigenvalues were determined to have negative real part provided condition (2) holds (so $L^*$, $P^*$, and $A^*$ are positive). The Routh-Hurwitz criteria can also be applied to the lower $4 \times 4$ block of the Jacobian matrix. Again, a necessary condition for stability is that all coefficients of the characteristic equation have the same sign. All coefficients for the characteristic equation for the lower $4 \times 4$ block are strictly
positive except for the constant coefficient, $a_0$, which is negative if the following condition holds:

$$c\alpha L^* \left( \frac{g_{P_L}}{g_{P_L} + \mu_{P_L}} \right) \left( \frac{g_{P_P}}{g_{P_P} + \mu_{P_P}} \right) \left( \frac{1}{\mu_{P_A}} \right) > 1.$$  

(9)

When this condition, also known as the invasion criteria, is met, the host-only equilibrium (7) is unstable and parasitoids are able to grow from low initial numbers when introduced into a stable host population. Biologically, condition (9) is met when the total number of offspring produced per adult parasitoid that survive to adulthood exceeds one, allowing the parasitoid population to persist.

The third equilibrium is the coexistence equilibrium (8), where both the host and parasitoid coexist. The equilibrium values at this steady state are as follows:

$$L^* = \frac{\mu_{P_A} (g_{P_L} + \mu_{P_L}) (g_{P_L} + \mu_{P_L})}{c\alpha g_{P_L} g_{P_L}},$$

$$P^* = \frac{g_L \mu_{P_A} (g_{P_L} + \mu_{P_L}) (g_{P_L} + \mu_{P_L})}{c\alpha g_{P_L} g_{P_L} g_{P_P}},$$

$$A^* = \frac{g_L \mu_{P_A} (g_{P_L} + \mu_{P_L}) (g_{P_L} + \mu_{P_L})}{c\alpha g_{P_L} g_{P_L} \mu_A},$$

$$P_E^* = \frac{c\alpha L^* P_A^*}{g_{P_L}},$$

$$P_L^* = \frac{\mu_{P_A} (g_{P_L} + \mu_{P_L})}{g_{P_L} g_{P_P}} P_A^*,$$

$$P_P^* = \frac{\mu_{P_A}}{g_{P_P}} P_A^*,$$

$$P_A^* = \left( \frac{1}{\alpha} \right) \left( \frac{b g_L - \mu_A (g_L + \mu_L)}{\mu_A} - \frac{b}{A^*} \right).$$

When the extinction and host-only equilibria are unstable, the coexistence equilibrium (8) may be stable, or the hosts and parasitoids may also enter into a limit cycle; this has been observed to occur in simulations for certain parameter values, and has also been seen in previous models such as the Nicholson and Bailey model (Godfray & Shimada, 1999; Mills & Getz, 1996; Nicholson & Bailey, 1935).

2.3 | Host-parasitoid-hyperparasitoid model

In this section, we incorporate two competing hyperparasitoid species to produce the full host-parasitoid-hyperparasitoid model (schematic in Figure 1). One species of hyperparasitoid attacks the larval stage of the parasitoid, and the other species attacks the pupal stage of the parasitoid (recall that $P_L$ includes both parasitoid prepupae and pupae). We model two stages (juvenile and adult) for each hyperparasitoid species. $H_J$ represents the density of larvae-attacking hyperparasitoid juveniles and $H_A$ represents the density of larvae-attacking hyperparasitoid adults. Similarly, $\hat{H}_J$ and $\hat{H}_A$ are the densities of the pupae-attacking hyperparasitoid juveniles and adults, respectively.
Larvae-attacking hyperparasitoid adults are assumed to attack parasitoid larvae at rate $\beta_L$. Larvae-attacking hyperparasitoid juveniles emerge from eggs oviposited into parasitoid larvae. The hyperparasitoid juveniles will mature into adults of the same species at rate $g_{HJ}$, and incur mortality at rate $\mu_{HJ}$. Note that this mortality rate incorporates both natural mortality as well as any increased mortality due to nicotine. Larvae-attacking hyperparasitoid adults have mortality rate $\mu_{HA}$. Pupae-attacking hyperparasitoid adults oviposit their eggs into the pupal stage of the parasitoid at rate $\beta_{P}$, producing pupae-attacking hyperparasitoid juveniles. These larvae will mature into hyperparasitoid adults at rate $g_{\hat{H}J}$, with mortality rate $\mu_{\hat{H}J}$. Pupae-attacking hyperparasitoid adults incur mortality at rate $\mu_{\hat{H}A}$.

Once the hyperparasitoid equations are coupled with model (5) (where the $P_L$ and $P_P$ equations have been modified to include hyperparasitism) we obtain the full host-parasitoid-hyperparasitoid model:

\[
\begin{align*}
\frac{dL}{dt} &= b \left( 1 - \frac{L}{K} \right) A - \mu_L L - g_{L} L - \alpha P_A L \\
\frac{dP}{dt} &= g_{L} L - g_{P} P \\
\frac{dA}{dt} &= g_{P} P - \mu_A A \\
\frac{dP_E}{dt} &= c\alpha P_A L - g_{P_E} P_E \\
\frac{dP_L}{dt} &= g_{P_L} P_L - g_{P_P} P_L - \mu_{P_L} P_L - \beta_L H_A P_L \\
\frac{dP_P}{dt} &= g_{P_P} P_L - g_{P_P} P_P - \mu_{P_P} P_P - \beta_P \hat{H}_A P_P \\
\frac{dP_A}{dt} &= g_{P_P} P_P - \mu_{P_A} P_A \\
\frac{dH}{dt} &= \beta_L H_A P_L - g_{H_L} H_J - \mu_{H_L} H_J \\
\frac{dH_A}{dt} &= g_{H_L} H_J - \mu_{H_A} H_A \\
\frac{d\hat{H}}{dt} &= \beta_P \hat{H}_A P_P - g_{\hat{H}_L} \hat{H}_J - \mu_{\hat{H}_L} \hat{H}_J \\
\frac{d\hat{H}_A}{dt} &= g_{\hat{H}_L} \hat{H}_J - \mu_{\hat{H}_A} \hat{H}_A
\end{align*}
\]

There are six equilibrium points for this model:

Extinction:

\[(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)\]  \hspace{1cm} (11)

Host only:

\[(L^*, P^*, A^*, 0, 0, 0, 0, 0, 0, 0, 0, 0)\]  \hspace{1cm} (12)

Host and parasitoid:

\[\left( L^*, P^*, A^*, P_E^*, P_L^*, P_P^*, P_A^*, 0, 0, 0, 0 \right)\]  \hspace{1cm} (13)
Host, parasitoid, and larvae-attacking hyperparasitoid:

\[
\begin{pmatrix}
L^*, P^*, A^*, P_E^*, P_L^*, P_P^*, P_{A^*}, H_{J^*}, H_{A^*}, 0, 0
\end{pmatrix}
\]  \hspace{1cm} (14)

Host, parasitoid, and pupae-attacking hyperparasitoid:

\[
\begin{pmatrix}
L^*, P^*, A^*, P_E^*, P_L^*, P_P^*, P_{A^*}, 0, 0, \hat{H}_{J^*}, \hat{H}_{A^*}
\end{pmatrix}
\]  \hspace{1cm} (15)

Coexistence of host, parasitoid, and both hyperparasitoids:

\[
\begin{pmatrix}
L^*, P^*, A^*, P_E^*, P_L^*, P_P^*, P_{A^*}, H_{J^*}, H_{A^*}, \hat{H}_{J^*}, \hat{H}_{A^*}
\end{pmatrix}
\]  \hspace{1cm} (16)

There are no equilibria where parasitoids and/or hyperparasitoids are present at positive levels in the absence of the host, since the host is necessary for these populations to survive. Similarly, there are no equilibria where hyperparasitoids persist in the absence of parasitoids. We will focus our analysis of this model on the stability of the three equilibria containing hyperparasitoids: two exclusion equilibria where only one hyperparasitoid species persists (14 and 15), and the coexistence equilibrium where both hyperparasitoid populations persist (16).

2.3.1 | Invasion criteria

Here we derive criteria for when one species of hyperparasitoid can invade the system with the other hyperparasitoid species at equilibrium. The species that is established is referred to as the “resident” while the species being introduced is referred to as the “invader.” If a resident population is at an

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**FIGURE 1** Schematic for Model (10). Solid lines indicate maturation to an older stage or reproduction. Dashed lines indicate parasitism, with $\alpha$, $\beta_L$, and $\beta_P$ representing the attack rates of primary parasitoid adults on host larvae, larvae-attacking hyperparasitoid adults on parasitoid larvae, and pupae-attacking hyperparasitoid adults on parasitoid pupae, respectively. Dotted lines indicate the production of eggs/juveniles resulting from parasitism. Larvae-attacking hyperparasitoid juveniles (circled in red) suffer increased mortality when nicotine is present.
equilibrium that is unstable, then when a small number of invaders is introduced their population can grow. This may result in either coexistence or extinction of the resident species (Murdoch et al., 2013), with coexistence occurring when both species can invade the other.

If larvae-attacking hyperparasitoids are the resident species and the system is at equilibrium (14), the criterion for pupae-attacking hyperparasitoids to be able to invade is:

\[
\beta_p \left( \frac{g_{H_P}}{g_{H_L} + \mu_{H_L}} \right) \left( \frac{1}{\mu_{H_L}} \right) P_p^* > 1
\]  

(17)

where \(P_p^* = \frac{g_{H_P} \mu_{H_L} (g_{H_P} + \mu_{H_P})}{g_{H_L} (g_{H_P} + \mu_{H_P})} \) is the equilibrium density of parasitoid pupae. If each individual from the invading species can produce more than one offspring that survives to become an adult over its lifetime, then it is possible for the species to invade. Total offspring for the pupae-attacking hyperparasitoid is determined by the product of the successful oviposition rate, \(\beta_p\), equilibrium density of parasitoid pupae, \(P_p^*\), and the expected lifespan of the hyperparasitoid, \(\frac{1}{\mu_{H_L}}\). The total number of offspring is then multiplied by the probability of survival to adulthood: \(\frac{g_{H_P}}{g_{H_L} + \mu_{H_L}}\). Note the total number of offspring produced by the invading species is dependent on the density of their host species at the stage the hyperparasitoid attacks, in this case the pupal stage of the primary parasitoid, \(P_p^*\). We remark that this density is also dependent on properties of the resident species that attacks an earlier stage of the primary parasitoid.

Substituting \(P_p^*\) into (17) with all parameters for the hyperparasitoid populations equal results in the condition:

\[
g_{P_L} \left( \frac{1}{g_{P_p} + \mu_{P_p}} \right) > 1.
\]  

(18)

Therefore pupae-attacking hyperparasitoids can invade a population of larvae-attacking hyperparasitoids if the maturation rate of the primary parasitoid larvae, \(g_{P_L}\), is greater than the combined rate at which parasitoid pupae leave the stage, either through maturation \(g_{P_p}\) or death \(\mu_{P_p}\). We note that condition (18) requires the pupal stage of the primary parasitoid be longer than the larval stage.

When nicotine is present it will increase the mortality rate of the larvae-attacking hyperparasitoid juveniles, \(\mu_{H_I}\). The only place this parameter appears in the invasion criteria (17) is in the numerator of \(P_p^*\). By increasing (or decreasing) \(\mu_{H_I}\) we increase (or decrease) the left-hand side of the invasion criteria (17). Therefore intensifying the effects of nicotine on the larvae-attacking hyperparasitoid resident makes it easier for the pupae-attacking hyperparasitoid to invade. Increasing the successful oviposition rate of the pupae-attacking hyperparasitoid relative to the larvae-attacking hyperparasitoid will also help to satisfy the invasion criteria.

Next we consider the case where pupae-attacking hyperparasitoids are the resident species, at equilibrium (15). The condition for larvae-attacking hyperparasitoids to be able to invade a resident population of pupae-attacking hyperparasitoids is:

\[
\beta_L \left( \frac{g_{H_L}}{g_{H_P} + \mu_{H_P}} \right) \left( \frac{1}{\mu_{H_P}} \right) P_p^* > 1
\]  

(19)
where
\[
P^*_{L} = \frac{\left( (b_{g_{L}} - \mu_{A}(g_{L} + g_{L}^*)\beta_{P}\mu_{P_{A}} - \mu_{H_{b}} g_{P_{A}} g_{R_{A}}\alpha)g_{H_{b}} - \mu_{H_{b}} g_{P_{A}} g_{R_{A}}\alpha \mu_{H_{E}} \right) c K (g_{H_{b}} + \mu_{H_{E}}) \mu_{H_{E}} g_{P_{A}} g_{R_{A}}\alpha}{\beta_{P}^{2} b_{g_{L}} g_{H_{b}}^{2} \mu_{P_{A}}^{2} (g_{P_{A}} + \mu_{P_{A}})}.\]

Again, we can interpret the left-hand side of condition (19) biologically as the total number of new adults produced by each larvae-attacking hyperparasitoid adult over its lifetime. Note that the effect of increasing \( \mu_{H_{b}} \) is reversed in the larval-attacker invasion criteria compared to the pupae-attacker invasion criteria. The mortality rate of the larvae-attacking hyperparasitoid juveniles, \( \mu_{H_{L}} \), is now in the denominator of the left-hand side of (19) and when the parameter is increased to simulate stronger effects of nicotine it will decrease the left-hand side, making it harder to reach the threshold of one required for invasion, and therefore lowering the ability of the larvae-attacking hyperparasitoids to invade a system where the pupae-attacking hyperparasitoids are established.

### 3 | MECHANISMS FOR COEXISTENCE

From Section 2.3.1, we know that increasing the death rate of the larva-attacking hyperparasitoid juveniles (\( \mu_{H_{L}} \)) due to nicotine will increase the ability of the pupae-attacking hyperparasitoids to invade a system where larva-attacking hyperparasitoids are established. Increasing the attack rate of the pupae-attacking hyperparasitoid (\( \beta_{P} \)) relative to the larva-attacking hyperparasitoid (\( \beta_{L} \)) can also provide an advantage for the pupae-attacking hyperparasitoid and help the species to persist. In this section, we further explore the effect of \( \mu_{H_{L}} \) and \( \beta_{P} \) on the dynamics of model (10), focusing on the hyperparasitoid populations.

Baseline parameter values for hosts, parasitoids, and hyperparasitoids are given in Table 1. Parameters are either estimated from the literature or reasonable values are assumed. Mortality rates were calculated from estimates on the percent of individuals that successfully reached the next stage or expected lifespan. For host pupae and parasitoid eggs, these stages are not subject to parasitism and we make the simplifying assumption that their mortality rates are zero; that is, all individuals mature into host adults or parasitoid larvae respectively. Larva-attacking hyperparasitoid parameters are taken to be the same as those for pupae-attackers. We note that under the parameter values in Table 1, the positive coexistence equilibrium of the Host-Parasitoid Model (8) is stable. Also, with these parameters and in the absence of nicotine (\( \mu_{H_{L}} = \mu_{H_{E}} = 0.016 \)), larva-attacking hyperparasitoids exclude pupae-attacking hyperparasitoids in the full Host-Parasitoid-Hyperparasitoid Model (10) as shown in Figure 2. Hosts, primary parasitoids, and larva-attacking hyperparasitoids all reach a positive steady state while pupae-attacking hyperparasitoids go extinct.

Increasing \( \mu_{H_{L}} \) relative to \( \mu_{H_{E}} \) causes equilibrium (14) (hosts, parasitoids, and larva-attacking hyperparasitoids) to lose stability. As \( \mu_{H_{L}} \) is increased from a baseline of 0.016, equilibrium dynamics change from exclusion of pupae-attacking hyperparasitoids, to coexistence of the hyperparasitoid species, to exclusion of the larva-attacking hyperparasitoids (see Figure 3a). Increasing \( \mu_{H_{L}} \) further has no impact on the model dynamics after the larva-attacking hyperparasitoids are extinct.

Differences in the rate of successful oviposition between hyperparasitoid species can also affect the outcome of competition. If pupae-attacking hyperparasitoids have an increased rate of successful oviposition relative to the larva-attacking hyperparasitoids, for example if \( \beta_{L} = 0.00002 \) and
then the species can coexist on a nicotine-free plant (see Figure 3b). Increasing $\mu_{HJ}$ from the nicotine-free baseline will continue to increase the total density of the pupae-attacking hyperparasitoid and decrease the total density of the larvae-attacking hyperparasitoid until the latter goes extinct. Exclusion of the larvae-attacking hyperparasitoid occurs at a lower value of $\mu_{HJ}$ when $\beta_P$ is increased relative to $\beta_L$.

### Table 1 Baseline parameter values for hosts, parasitoids, and hyperparasitoids

| Parameter | Meaning | Estimated value | Source |
|-----------|---------|-----------------|--------|
| $b$       | Adult per capita birthrate | 30 | Madden and Chamberlin (1945) |
| $K$       | Larval density dependent factor | 10 | Assumed |
| $g_L$     | Larval maturation rate | $1/20 \text{ days}^{-1}$ | Madden and Chamberlin (1945) |
| $g_P$     | Pupal maturation rate | $1/22 \text{ days}^{-1}$ | Reinecke et al. (1980) |
| $\mu_L$   | Larval mortality rate | $0.0125 \text{ days}^{-1}$ | Kingsolver (2007) |
| $\mu_A$   | Adult mortality rate | $1/10 \text{ days}^{-1}$ | Madden and Chamberlin (1945) |
| $c$       | Clutch size | 49.85 | Barbosa et al. (1986) |
| $\alpha$  | Oviposition rate | 0.002 | Assumed |
| $g_{PE}$  | Egg maturation rate | $1/2 \text{ days}^{-1}$ | Fulton (1940); Lawrence (2010) |
| $g_{PL}$  | Larval maturation rate | $1/9.7 \text{ days}^{-1}$ | Barbosa et al. (1986) |
| $g_{PP}$  | Pupal maturation rate | $1/6.8 \text{ days}^{-1}$ | Barbosa et al. (1986) |
| $\mu_{PL}$| Larval mortality rate | $0.016 \text{ days}^{-1}$ | Barbosa et al. (1986) |
| $\mu_{PP}$| Pupal mortality rate | $0.028 \text{ days}^{-1}$ | Barbosa et al. (1986) |
| $\mu_{PA}$| Adult mortality rate | $1/4.6 \text{ days}^{-1}$ | Dhammi (2010) |

| Parameter | Meaning | Estimated value | Source |
|-----------|---------|-----------------|--------|
| $\beta_L$| Oviposition rate (larvae-attacking) | 0.00002 | Assumed |
| $\beta_P$| Oviposition rate (pupae-attacking) | 0.00002 | Assumed |
| $g_{Hb}$ | Juvenile maturation rate (larvae-attacking) | $1/14 \text{ days}^{-1}$ | Assumed |
| $g_{Hb}$ | Juvenile maturation rate (pupae-attacking) | $1/14 \text{ days}^{-1}$ | McNeil and Rabb (1973) |
| $\mu_{Hb}$| Juvenile mortality rate (larvae-attacking) | $\geq 0.016 \text{ days}^{-1}$ | Assumed |
| $\mu_{Hb}$| Juvenile mortality rate (pupae-attacking) | 0.016 days$^{-1}$ | Assumed |
| $\mu_{Ha}$| Adult mortality rate (larvae-attacking) | $1/21 \text{ days}^{-1}$ | Assumed |
| $\mu_{Ha}$| Adult mortality rate (pupae-attacking) | $1/21 \text{ days}^{-1}$ | McNeil and Rabb (1973) |

Note: Oviposition rates are per capita number of successful ovipositions (on host larvae, parasitoid larvae, or parasitoid pupae) per parasitoid per day.
We next look at how changing the oviposition rate of the pupae-attacking hyperparasitoid relative to the larvae-attacking hyperparasitoid affects dynamics, for varying levels of nicotine. Let \( k \) be the ratio of the successful attack rates of the pupae-attacking hyperparasitoids and the larvae-attacking hyperparasitoids, so \( k = \frac{\beta_p}{\beta_l} \). On a plant with no nicotine, so \( \mu_H = \mu_H = 0.016 \), setting \( \beta_L = 0.00002 \) and increasing \( k \) (and therefore \( \beta_p \)) results in equilibrium (14) (exclusion of pupae-attacking hyperparasitoids) losing stability for \( k \) just below 2, when the oviposition success rate of the pupae-attacking hyperparasitoids is almost twice that of the larvae-attacking hyperparasitoids (see Figure 4a).

![Figure 2](image1.png)

**Figure 2** Time series of Model (10) with parameters as in Table 1 and \( \mu_H = 0.016 \). Initial conditions were 10 adult hosts, 10 adult primary parasitoids, and 10 adults of each hyperparasitoid species. Larvae-attacking hyperparasitoids exclude pupae-attacking hyperparasitoids.

We next look at how changing the oviposition rate of the pupae-attacking hyperparasitoid relative to the larvae-attacking hyperparasitoid affects dynamics, for varying levels of nicotine. Let \( k \) be the ratio of the successful attack rates of the pupae-attacking hyperparasitoids and the larvae-attacking hyperparasitoids, so \( k = \frac{\beta_p}{\beta_l} \). On a plant with no nicotine, so \( \mu_H = \mu_H = 0.016 \), setting \( \beta_L = 0.00002 \) and increasing \( k \) (and therefore \( \beta_p \)) results in equilibrium (14) (exclusion of pupae-attacking hyperparasitoids) losing stability for \( k \) just below 2, when the oviposition success rate of the pupae-attacking hyperparasitoids is almost twice that of the larvae-attacking hyperparasitoids (see Figure 4a).

![Figure 3](image2.png)

**Figure 3** The total population size (juveniles plus adults) of larvae-attacking and pupae-attacking hyperparasitoids at equilibrium is plotted against the bifurcation parameter \( \mu_H \), the mortality rate of larvae-attacking hyperparasitoid juveniles. Other parameters are as in Table 1, with initial conditions as in Figure 2. (a) \( \beta_L = \beta_p = 0.00002 \); (b) \( \beta_L = 0.00002, \beta_p = 0.00004 \)
There is a large range of $k$ for which the hyperparasitoids coexist, either at equilibrium or via stable limit cycles. Increasing $k$ far enough will eventually lead to the exclusion of the larvae-attacking parasitoids ($\approx k \approx 5.5$).

On a nicotine-producing plant with $\mu_{Hb} = 0.08$, we see similar dynamics when varying the attack rates of the hyperparasitoid species (see Figure 4b). Larvae-attacking parasitoids exclude pupae-attacking parasitoids for low values of $k$ (note there is coexistence here for $k \approx 1$). The hyperparasitoids coexist at equilibrium (16) for intermediate values of $k$ (note we no longer see oscillations), and for $k$ high enough larvae-attacking parasitoids are excluded by pupae-attacking parasitoids. This exclusion threshold occurs at a lower value of $k$ ($k \approx 3$) than in the nicotine-free system. The equilibrium population size for pupae-attacking hyperparasitoids increases with $k$ (and $\beta_p$) until larvae-attacking hyperparasitoids go extinct. Continuing to increase $k$ once pupae-attacking hyperparasitoids have excluded larvae-attacking hyperparasitoids has a detrimental effect on the total population size of the pupae-attacking hyperparasitoid due to decreased numbers of primary parasitoids as a result of the higher attack rate.

**FIGURE 4** The total population size (juveniles plus adults) of larvae-attacking and pupae-attacking hyperparasitoids is plotted against $k$, the ratio of successful oviposition rate of pupae-attacking hyperparasitoid adults to the successful oviposition rate of larvae-attacking hyperparasitoid adults. Here $\beta_L = 0.00002$, other parameters are as in Table 1, and initial conditions are as in Figure 2. (a) $\mu_{Hb} = \mu_{Hb} = 0.016$; (b) $\mu_{Hb} = 0.08$, $\mu_{Hb} = 0.016$; (c) $\mu_{Hb} = 0.36$, $\mu_{Hb} = 0.016$.
For $\mu = 0.08$, increasing $k$ (or, equivalently, $\beta_p$) always results in either constant or decreased equilibrium population sizes for the larvae-attacker, as we change from the larvae-attacker excluding the pupae-attacker, to coexistence, and finally to the pupae-attacker excluding the larvae-attacker. On a plant with even higher levels of nicotine, so $\mu = 0.36$, both species go extinct if $k$ (or, equivalently, $\beta_p$) is too low. As $k$ is increased from zero, the pupae-attacking hyperparasitoids are the first to be able to persist ($k \approx 0.38$). Continuing to increase $k$ further results in a small region of coexistence ($k \approx 0.42 - 0.73$), after which the pupae-attacking hyperparasitoid again excludes the larvae-attacking hyperparasitoid. We note the latter region includes the scenario of equal attack rates ($k = 1$).

Figure 5 shows the outcome of competition between the two hyperparasitoid species as the mortality rate due to nicotine, $\mu$, and the attack rate of the pupae-attacking hyperparasitoid, $\beta_p$, vary. If $\beta_p$ is sufficiently low and $\mu$ is sufficiently high, neither species of hyperparasitoid can persist. For low values of $\beta_p$ and $\mu$, larvae-attacking hyperparasitoids exclude pupae-attacking hyperparasitoids. As either parameter is increased, pupae-attacking hyperparasitoids gain a fitness advantage over their competitor, either by incurring a lower mortality rate or increased attack rate.

**4 | EVOLUTION OF PLANT CHOICE**

In this section we use an evolutionary game theoretic approach to investigate how plant-specific oviposition rates of competing larvae-attacking and pupae-attacking hyperparasitoid species may evolve in an environment containing both nicotine-free plants (e.g., tomato) and plants that produce nicotine (e.g., tobacco), referred to here as nicotine-producing plants. Although *C. congregata* adults can fly, we make the simplifying assumptions that each plant type has an associated host and primary parasitoid population that does not utilize the alternative plant type. Both species of hyperparasitoid have access to both types of plants, with overall oviposition rates $\beta_L$ and $\beta_p$ for the larvae-attacking and pupae-attacking hyperparasitoids, respectively. We define the strategy parameter $p$ to be the fraction of attacks by larvae-attacking hyperparasitoids that occur on parasitized hosts on nicotine-free plants, and similarly the strategy parameter $q$ is the fraction of attacks by pupae-attacking hyperparasitoids that occur on parasitized hosts on nicotine-producing plants.
nicotine-free plants. It follows that $1 - p$ and $1 - q$ are the fraction of attacks made by larve- and pupae-attacking hyperparasitoids, respectively, on parasitized hosts on nicotine-producing plants. A model schematic is shown in Figure 6 and the equations for the two-plant model are:

$$
\begin{align*}
\frac{dL}{dt} &= b\left(1 - \frac{L}{K}\right)A - \mu_L L - g_L L - \alpha P_A L \\
\frac{dP}{dt} &= g_L L - g_P P \\
\frac{dA}{dt} &= g_P P - \mu_A A \\
\frac{dP_E}{dt} &= c\alpha P_A L - g_{P_E} P_E \\
\frac{dP_l}{dt} &= g_{P_l} P_E - g_{P_l} P_l - \mu_{P_l} P_l - \beta_L H_A P_l \\
\frac{dP_p}{dt} &= g_{P_p} P_l - g_{P_p} P_p - \mu_{P_p} P_p - q\beta_P H_A P_p \\
\frac{dP_A}{dt} &= g_{P_A} P_p - \mu_{P_A} P_A \\
\frac{dH_l}{dt} &= p\beta_L H_A P_l - g_{H_l} H_l - \mu_{H_l} H_l \\
\frac{dH_A}{dt} &= q\beta_P H_A P_p - g_{H_A} H_A - \mu_{H_A} H_A \\
\frac{dL^+}{dt} &= b\left(1 - \frac{L^+}{K}\right)A^+ - \mu_L L^+ - g_L L^+ - \alpha P_A^+ L^+ \\
\frac{dP^+}{dt} &= g_L L^+ - g_P P^+ \\
\frac{dA^+}{dt} &= g_P P^+ - \mu_A A^+ \\
\frac{dP_E^+}{dt} &= c\alpha P_A^+ L^+ - g_{P_E} P_E^+ \\
\frac{dP_l^+}{dt} &= g_{P_l} P_E^+ - g_{P_l} P_l^+ - \mu_{P_l} P_l^+ - (1 - p)\beta_L H_A P_l^+ \\
\frac{dP_p^+}{dt} &= g_{P_p} P_l^+ - g_{P_p} P_p^+ - \mu_{P_p} P_p^+ - (1 - q)\beta_P H_A P_p^+ \\
\frac{dP_A^+}{dt} &= g_{P_A} P_p^+ - \mu_{P_A} P_A^+ \\
\frac{dH_l^+}{dt} &= (1 - p)\beta_L H_A P_l^+ - g_{H_l} H_l^+ - \mu_{H_l} H_l^+ \\
\frac{dH_A^+}{dt} &= (1 - q)\beta_P H_A P_p^+ - g_{H_A} H_A^+ - \mu_{H_A} H_A^+ \\
\frac{dH_l}{dt} &= g_{H_l} (H_l + H_l^+) - \mu_{H_l} H_l \\
\frac{dH_A}{dt} &= g_{H_A} (H_A + H_A^+) - \mu_{H_A} H_A. 
\end{align*}
$$

(20)
where all variables and parameters are defined as in the single plant model and a “+” denotes populations on the nicotine-producing plant.

The subset of the two-plant model (20) representing the dynamics of the larva-attacking hyperparasitoids is given by $x' = G(p, P_L, P_L^+)x$, where $x = \begin{bmatrix} H_L & H_L^+ & H_A \end{bmatrix}^T$, and the “fitness” matrix, which determines net reproductive output, is

$$G(p, P_L, P_L^+) = \begin{bmatrix} -g_{H_L} - \mu_{H_L} & 0 & p\beta_L P_L \\ 0 & -g_{H_L} - \mu_{H_L}^+ & (1 - p)\beta_L P_L^+ \\ g_{H_L} & g_{H_L} & -\mu_{H_A} \end{bmatrix}. \quad (21)$$

The subset of (20) representing the pupae-attacking hyperparasitoids is $\dot{x}' = \dot{G}(q, P_P, P_P^+)\dot{x}$, where $\dot{x} = \begin{bmatrix} H_L & \dot{H}_L^+ & \dot{H}_A \end{bmatrix}^T$, and

$$\dot{G}(q, P_P, P_P^+) = \begin{bmatrix} -g_{H_L} - \mu_{H_L} & 0 & q\beta_P P_P \\ 0 & -g_{H_L} - \mu_{H_L}^+ & (1 - q)\beta_P P_P^+ \\ g_{H_L} & g_{H_L} & -\mu_{H_A} \end{bmatrix}. \quad (22)$$

The strategies $p$ and $q$ are taken to be hereditary traits that evolve according to the differential equations.

**FIGURE 6** Schematic for two plant model (20). Solid lines indicate maturation to an older stage or reproduction. Dashed lines indicate parasitism, with $\alpha$, $p\beta_L$, $(1 - p)\beta_L$, $q\beta_P$, and $(1 - q)\beta_P$, representing the attack rates of primary parasitoid adults on host larvae on each plant, larva-attacking hyperparasitoid adults on parasitoid larvae on the nicotine-free plant, larva-attacking hyperparasitoid adults on parasitoid larvae on the nicotine-producing plant, pupae-attacking hyperparasitoid adults on parasitoid pupae on the nicotine-free plant, and pupae-attacking hyperparasitoid adults on parasitoid pupae on the nicotine-producing plant, respectively. Dotted lines indicate the production of eggs/juveniles resulting from parasitism. Larvae-attacking hyperparasitoid juveniles (circled in red) suffer increased mortality on the plant producing nicotine.
where $G$ and $\hat{G}$ are the fitness functions and denote the spectral radius of $G$ and $\hat{G}$, respectively (Vincent & Brown, 2005). The terms $\sigma_p^2$ and $\sigma_q^2$ are the variances of the traits in each population, which determine the speed of evolution. Modeling evolution simultaneously with population dynamics, the full system includes the two-plant model (20) with the equations for trait evolution (23) and (24). Equilibria of this model that optimize fitness will indicate evolutionarily stable strategies.

We find that if the death rate of larvae-attacking hyperparasitoids is the same on plants with and without nicotine ($\mu_{H_L} = \mu_{H_A}$), then the evolutionary equilibrium for the strategies is $p^* = q^* = 0.5$, or equal attack rates on both plants for both species. When the larvae-attacking parasitoids are adversely affected by nicotine this is no longer the outcome of evolution. Figure 7 shows how the strategies $p$ and $q$ evolve over time from an initial condition of $p = q = 0.5$ (both species using both plants equally) for the case where there is an increased death rate due to nicotine for the larvae-attacking hyperparasitoids on the nicotine-producing plant only ($\mu_{H_L} = 0.08, \mu_{H_A} = 0.016$), and pupae-attacking hyperparasitoids have a higher attack rate than larvae-attacking hyperparasitoids ($\beta_L = 0.00002, \beta_P = 0.00004$). Recall these parameters result in a stable coexistence equilibrium for the single plant model (10). The evolutionary equilibrium values of the strategies for the two plant model are now $p^* = 0.778$ and $q^* = 0.146$. That is, larvae-attacking hyperparasitoids attack parasitoid larvae on nicotine-free plants 77.8% of the time, and the other 22.2% of attacks are on parasitoid larvae on nicotine-producing plants. Pupa-attacking hyperparasitoids only attack parasitoid pupae on nicotine-free plants 14.6% of the time, and the other 85.4% of attacks are on parasitoid pupae on the nicotine-producing plant.

To compare the corresponding hyperparasitoid population dynamics for two sets of strategies, model (20) was simulated for the arbitrary initial condition $p = q = 0.5$ as well as the evolutionary equilibrium $p = p^* = 0.778, q = q^* = 0.146$. For $p = q = 0.5$, the equilibrium population sizes are $\left(\hat{H}_L, \hat{H}_L^+, \hat{H}_A, \hat{H}_R, \hat{H}_R^+, \hat{H}_A\right) = (4682, 2703, 11079, 1439, 1439, 4318)$.
For $p = 0.778$ and $q = 0.146$, the equilibrium population sizes are $(H_J, H_J^+, H_A, \hat{H}_J, \hat{H}_J^+, \hat{H}_A) = (5747, 1640, 11080, 519, 3035, 5330)$. While the number of larvae-attacking hyperparasitoid adults remains almost constant, there are more larvae on the nicotine-free plant and fewer on the nicotine-producing plant for the equilibrium values of $p$ and $q$ compared to when $p = q = 0.5$. The adult pupae-attacking hyperparasitoid population is 23% larger for $p = p^*$ and $q = q^*$ compared to when $p = q = 0.5$, with fewer larvae on the nicotine-free plant and larger populations on the nicotine-producing plant.

Figure 8 shows how strategy equilibria, along with the corresponding steady state population dynamics, change as the death rate of larvae-attacking hyperparasitoid juveniles on the nicotine-producing plant ($\mu_{HJ}$) increases. Results are shown for the pupae-attacking hyperparasitoid oviposition rate equal to that of the larvae-attacking hyperparasitoids ($\beta_p = 0.00002$) as well as twice as large ($\beta_p = 0.00004$).

If larvae-attacking and pupae-attacking hyperparasitoids have equal attack rates ($\beta_L = \beta_p = 0.00002$), then larvae-attackers will exclude pupae-attackers on both plants when either both plants are nicotine-free or the effects of nicotine on the nicotine-producing plant are small ($\mu_{HJ} \mu_{HJ}^* < 0.07$). Larvae-attacking hyperparasitoids use both plants equally ($p^* = 0.5$) if $\mu_{HJ} = \mu_{HJ}^*$. As $\mu_{HJ}$ increases, fewer larvae-attacking hyperparasitoid juveniles can survive on the nicotine-producing plant and $p^*$, the fraction of attacks on the nicotine-positive plant, increases slightly.

Once $\mu_{HJ}^*$ is large enough, both species of hyperparasitoids can coexist on the nicotine-producing plant. Since pupae-attacking hyperparasitoids can never persist on the nicotine-free

**FIGURE 8** Shown are the evolutionary equilibria for the larvae-attacker trait $p$ and the pupae-attacker trait $q$ as a function of $\mu_{HJ}^*$, increased death rate of larvae-attacking hyperparasitoid juveniles on the nicotine-producing plant, along with the corresponding equilibrium levels of larvae- and pupae-attacking hyperparasitoid populations. For the top row, $\beta_p = 0.00002$ and for the bottom row $\beta_p = 0.00004$. For all simulations $\beta_L = 0.00002$ and other parameters are as in Table 1.
plant, all of their attacks are on the nicotine-producing plant \((q^* = 0)\). As \(\mu_{H_l^*}\) increases, the equilibrium number of larvae-attacking hyperparasitoid juveniles on the nicotine-free plant is unchanged, equilibrium population sizes of the pupae-attacking hyperparasitoid on the nicotine-producing plant increase and equilibrium population sizes of the larvae-attacking hyperparasitoid juveniles on the nicotine-producing plant decrease. This results in a decrease in the adult larvae-attacking hyperparasitoid population size and an increase in the percentage of attacks on the nicotine-free plant, \(p^*\), as \(\mu_{H_l^*}\) increases.

Once \(\mu_{H_l^*}\) reaches a value of approximately 0.29 the larvae-attacking hyperparasitoids can no longer persist on the nicotine-producing plant, and 100% of their attacks are on the nicotine-free plant \((p = 1)\), resulting in complete spatial segregation of the species.

When \(\beta_L = 0.00002\) and \(\beta_p = 0.00004\), both hyperparasitoid species coexist in the absence of nicotine. Both species have equal attack rates \((p^* = q^* = 0.5)\) and population sizes on both plants if \(\mu_{H_l^*} = \mu_{H_p^*}\). Again, equilibrium population sizes on the nicotine-free plant are not affected by \(\mu_{H_l^*}\). As \(\mu_{H_l^*}\) increases, \(p^*\) increases and \(q^*\) decreases as the juvenile population sizes supported on the nicotine-producing plant decrease for the larvae-attacking hyperparasitoid and increase for the pupae-attacking hyperparasitoid.

Once \(\mu_{H_l^*}\) exceeds a value of approximately 0.14, the effects of nicotine are too strong for the larvae-attacking hyperparasitoids to persist on the nicotine-producing plant, and 100% of their attacks are on the nicotine-free plant \((p = 1)\). Here both species still coexist on the nicotine-free plant, but the nicotine-producing plant contains only pupae-attacking hyperparasitoids. Since pupae-attacking hyperparasitoids are not affected by nicotine, \(p^*\) and \(q^*\) remain constant for any further increase in \(\mu_{H_l^*}\).

\section{DISCUSSION}

We have modeled a system of hosts, parasitoids, and two competing hyperparasitoids, incorporating trade-offs between the competing hyperparasitoid species that affect the population dynamics of the system and the outcome of competition between the hyperparasitoids. While the larvae-attacking hyperparasitoids have the advantage of attacking an earlier stage of the primary parasitoid, they incur a cost of increased mortality at the juvenile stage in the presence of nicotine. On the other hand, the pupae-attacking hyperparasitoids must wait for primary parasitoids to escape parasitism by larvae-attacking hyperparasitoids and make it past the larval stage to the pupal stage before they can attack, but they do not incur any cost due to nicotine. We find that larvae-attacking hyperparasitoids will exclude pupae-attacking hyperparasitoids in the absence of nicotine, when there are no differences in the species other than the stage of the parasitoid in which they deposit their eggs. Similarly, Briggs found that two parasitoid species attacking different life-stages of their host, eggs, and larvae, could not coexist when larvae-attacking parasitoids could not attack already-parasitized hosts \((Briggs, 1993)\). We find that the two hyperparasitoid species can coexist when the cost to the larvae-attackers, due to nicotine, becomes high enough, or when pupae-attackers have an additional competitive advantage such as an increased oviposition rate.

With equal attack rates among hyperparasitoid species, coexistence can be attained if the cost of increased mortality due to nicotine for the larvae-attacking hyperparasitoid outweighs the benefit of attacking the earlier stage of the parasitoid. As \(\mu_{H_l}\) increases, eventually a level is reached where the larvae-attackers can no longer exclude the pupae-attacking hyperparasitoids and coexistence is possible. This threshold value of \(\mu_{H_l}\) is lower when pupae-attacking
hyperparasitoids also have an additional advantage, such as increased attack rates on the parasitoid. If $\mu_{H}$ continues to increase, the cost of mortality becomes too great to overcome and eventually the larvae-attacking hyperparasitoid will not be able to persist. The mortality rate of larvae-attacking hyperparasitoid juveniles may depend on the concentrations of nicotine being produced by the plant and subsequently transferred by the host to the primary parasitoid. The impact of a given concentration of nicotine may also vary with species and how well-adapted they are to nicotine. Species with no prior exposure may incur greater costs than species that have had prior exposure and evolved adaptations.

Coexistence of larvae- and pupae-attacking hyperparasitoid species has been observed to occur on nicotine-free plants. For example, both larvae- and pupae-attacking hyperparasitoids have been reared from cocoons from single broods of C. congregata collected from hosts that fed on nicotine-free plants in the field; however, only prepupa-attacking hyperparasitoids have been reared from C. congregata collected from M. sexta on tobacco (Karen Kester, personal communication). The advantage larvae-attacking hyperparasitoids gain from attacking the earlier stage of the parasitoid would prevent coexistence on a nicotine-free plant, given equal attack rates. However, coexistence can occur when the pupae-attacking species has the advantage of a higher successful rate of oviposition ($\beta_p > \beta_L$). The attack rates $\beta_L$ and $\beta_p$ are based on the success of the hyperparasitoids both in search and handling time. Ovipositing into the larva of the parasitoid through the cuticle of the host is a relatively rare trait among hyperparasitoids. Possibly, handling times are greater for larvae-attacking hyperparasitoids due to defensive behavior of the parasitized host. The hyperparasitoids can also coexist in the presence of nicotine; however, if the effects of nicotine become too strong, the larvae-attacking hyperparasitoid can no longer persist. As the successful attack rate of the pupae-attacking hyperparasitoids is increased relative to that of the larvae-attacking hyperparasitoids on a nicotine-producing plant, we see that exclusion of the larvae-attackers generally occurs for weaker effects of nicotine (lower values of $\mu_{H}$). The scenario where pupae-attacking hyperparasitoids have increased oviposition rates and larvae-attacking hyperparasitoid juveniles suffer increased mortality due to nicotine results in model dynamics consistent with observations: coexistence of both hyperparasitoid species on nicotine-free plants, and the absence of larvae-attacking hyperparasitoids on plants producing high enough levels of nicotine.

We note that our model has been parameterized so larvae-attacking hyperparasitoids can parasitize all internal instars of the larval stage of the primary parasitoid and pupae-attacking hyperparasitoids can parasitize both the prepupal and pupal stages. In reality, the stages that specific hyperparasitoids attack may be narrower. Future work includes extending the model to explicitly account for individual larval instars and the prepupal stage alone.

To further explore the impact of nicotine on hyperparasitoid diversity, we used evolutionary game theory to determine optimal oviposition strategies for both species when faced with the decision of whether to attack parasitoids on a plant with or without nicotine. When overall attack rates are equal between hyperparasitoid species, the optimal strategy for the pupae-attacking hyperparasitoid is to never attack any parasitoids on the nicotine-free plant. However, this limited diversity outcome is a result of the two species being unable to coexist with equal attack rates in the absence of nicotine, with pupae-attacking hyperparasitoids being excluded.

If pupae-attacking hyperparasitoids have an attack rate sufficiently greater than that of larvae-attacking hyperparasitoids, both species can coexist in the absence of nicotine. In the case that nicotine does not affect larvae-attacking hyperparasitoids, both plants are effectively the same and both species make 50% of their ovipositions on each plant. As mortality due to nicotine on the nicotine-producing plant, $\mu_{H}^*$, increases, larvae-attacking hyperparasitoids
make a greater percentage of ovipositions on the nicotine-free plant, and pupae-attacking hyperparasitoids increase the percentage of attacks on nicotine-producing plants. Eventually $\mu_{H^1}$ is increased high enough that larvae-attacking hyperparasitoids cannot persist on the nicotine-producing plant, and 100% of their ovipositions occur on the nicotine-free plant. At this point, the pupae-attacking hyperparasitoid is attacking parasitoids on both plants, so we observe both species on the nicotine-free plant but only pupae-attacking hyperparasitoids on the nicotine-producing plant.

Our model assumes that each plant has separate populations of hosts and primary parasitoids with equal parameter values. This assumption is reasonable for populations that are nicotine-adapted but may break down for populations that are not nicotine-adapted. For example, *C. congregata* associated with *M. sexta* on tobacco is unaffected by low concentrations of nicotine in the host diet that otherwise cause mortality in *C. congregata* associated with *M. sexta* on tomato (Kester & Barbosa, 1991). When offered tomato and tobacco plants simultaneously in the field, the tobacco-associated population parasitizes hosts on tobacco and tomato equally, whereas the tomato-associated population prefers hosts on tomato (Kester & Barbosa, 1994). Relaxing the assumption that all populations of the primary parasitoid are adapted to nicotine would introduce differences between the nicotine-producing and nicotine-free primary parasitoid populations and therefore alter the optimal distribution of hyperparasitoid attack rates between the two plants.

## 6 CONCLUSION

The dynamics of populations interacting over multiple trophic levels are complex and intertwined. Here we have shown that the result of competition between two hyperparasitoid species at the fourth trophic level can depend upon the presence or absence of a plant allelochemical, such as nicotine, at the primary trophic level. The hyperparasitoid attacking the earlier life-stage of the primary parasitoid is favored in the absence of nicotine, but coexistence of the hyperparasitoids is possible if the species attacking the later life-stage has an additional advantage such as a higher oviposition rate. The presence of nicotine at the primary trophic level also promotes coexistence of the hyperparasitoid species by increasing the mortality rate of the larvae-attacking hyperparasitoids. However, if the effects of nicotine are too strong, larvae-attacking hyperparasitoids will no longer be able to persist.

In a system with two hyperparasitoid species attacking parasitoids on two different plants, each species will distribute attacks between both plants equally if they are both nicotine-free, with coexistence on both plants possible when pupae-attacking hyperparasitoids have a higher attack rate. When one of the plants produces nicotine, however, this distribution is altered. As the levels of nicotine on one of the plants increases, larvae-attacking hyperparasitoids increase the fraction of their ovipositions on the nicotine-free plant, while pupae-attacking hyperparasitoids favor the nicotine-producing plant. When deleterious effects of nicotine on the larvae-attacking hyperparasitoid are high enough, this species attacks the nicotine-free plant exclusively, while the pupae-attacking hyperparasitoid continues to attack parasitoids on both plants. In this case the diversity of hyperparasitoid species is greater on nicotine-free plants compared to plants producing nicotine, consistent with observations.

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AUTHOR CONTRIBUTIONS

Mark Zimmerman: Conceptualization (equal), formal analysis (lead), methodology (equal), software (equal), and writing original draft (equal). David M. Chan: Formal analysis (supporting), writing original draft (supporting), and writing review and editing (supporting). Karen M. Kester: Conceptualization (equal), validation (lead), writing original draft (supporting), and writing review and editing (supporting). Rosalyn C. Rael: Methodology (equal), software (equal) and writing original draft (supporting). Suzanne L. Robertson: Conceptualization (equal), formal analysis (supporting), methodology (equal), software (equal), supervision (lead), writing original draft (equal), and writing review and editing (lead).

DATA AVAILABILITY STATEMENT

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

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REFERENCES

Barbosa, P., Saunders, J. A., Kemper, J., Trumbule, R., Olechno, J., & Martinat, P. (1986). Plant allelochemicals and insect parasitoids effects of nicotine on Cotesia congregata (Say) (Hymenoptera: Braconidae) and Hyposoter annulipes (Cresson) (Hymenoptera: Ichneumonidae). Journal of Chemical Ecology, 12(6), 1319–1328.

Beddington, J. R., & Hammond, P. S. (1977). On the dynamics of host-parasite-hyperparasite interactions. The Journal of Animal Ecology, 46, 811–821.

Bonsall, M. B., Hassell, M. P., & Asefa, G. (2002). Ecological trade-offs, resource partitioning, and coexistence in a host-parasitoid assemblage. Ecology, 83(4), 925–934.

Briggs, C. J. (1993). Competition among parasitoid species on a stage-structured host and its effect on host suppression. The American Naturalist, 141, 372–397.

Chowański, S., Adamski, Z., Marciniak, P., Rosiński, G., Büyükgüzel, E., Büyükgüzel, K., Falabella, P., ScraLo, L., Ventrella, E., Lelario, F. et al. (2016). A review of bioinsecticidal activity of solanaceae alkaloids. Toxins, 8(3), 60.

Comins, H. N., & Hassell, M. P. (1996). Persistence of multispecies host–parasitoid interactions in spatially distributed models with local dispersal. Journal of theoretical Biology, 183(1), 19–28.

Dharmi, A. (2010). Effect of imidacloprid on Cotesia congregata, an endoparasitoid of Manduca sexta, and its translocation from host to endoparasitoid (Master’s thesis). North Carolina State University.

Fulton, B. B. (1940). The hornworm parasite, Apanteles congregatus say and the hyperparasite, Hypopteromalus tabacum (Fitch). Annals of the Entomological Society of America, 33(2), 231–244.

Godfray, H. C. J., & Shimada, M. (1999). Parasitoids as model organisms for ecologists. Researches on Population Ecology, 41(1), 3–10.

Godfray, H. C. J. (1994). Parasitoids: Behavioral and evolutionary ecology (Vol. 67). Princeton University Press.

Harvey, J. A., Van Dam, N. M., Witjes, L., Soler, R., & Gols, R. (2007). Effects of dietary nicotine on the development of an insect herbivore, its parasitoid and secondary hyperparasitoid over four trophic levels. Ecological Entomology, 32(1), 15–23.

Hassell, M. P. (2000). Host-parasitoid population dynamics. Journal of Animal Ecology, 69(4), 543–566.

Hassell, M. P., & May, R. M. (1986). Generalist and specialist natural enemies in insect predator-prey interactions. The Journal of Animal Ecology, 55, 923–940.

Hogarth, W. L., & May, R. M. (1984). Interspecific competition in larvae between entomophagous parasitoids. The American Naturalist, 124(4), 552–560.
Holt, R. D., & Hochberg, M. E. (1998). The coexistence of competing parasites. Part II hyperparasitism and food chain dynamics. *Journal of Theoretical Biology, 193*(3), 485–495.

Ives, A. R. (1992). Continuous-time models of host-parasitoid interactions. *The American Naturalist, 140*(1), 1–29. https://doi.org/10.1086/285400

Kakehashi, N., Suzuki, Y., & Iwasa, Y. (1984). Niche overlap of parasitoids in host-parasitoid systems: Its consequence to single versus multiple introduction controversy in biological control. *Journal of Applied Ecology, 21*(1), 115–131. https://doi.org/10.2307/2403041

Kester, K. M., & Barbosa, P. (1991). Behavioral and ecological constraints imposed by plants on insect parasitoids: Implications for biological control. *Biological Control, 1*(2), 94–106.

Kester, K. M., & Barbosa, P. (1994). Behavioral responses to host foodplants of two populations of the insect parasitoid *Cotesia congregata* (Say). *Oecologia, 99*(1–2), 151–157.

Kingsolver, J. G. (2007). Variation in growth and instar number in field and laboratory *Manduca sexta*. *Proceedings of the Royal Society B: Biological Sciences, 274*(1612), 977–981.

Lawrence, S. E. (2010). *Feeding behaviors of larval Manduca sexta parasitized by Cotesia congregata* (Master’s thesis). State University of New York at Binghamton.

Madden, A. H., & Chamberlin, F. S. (1945). *Biology of the tobacco hornworm in the southern cigar-tobacco district* (Technical Report).

May, R. M., & Hassell, M. P. (1981). The dynamics of multiparasitoid-host interactions. *The American Naturalist, 117*(3), 234–261.

McNeil, J. N., & Rabb, R. L. (1973). Life histories and seasonal biology of four hyperparasites of the tobacco hornworm. *Manduca sexta* (Lepidoptera: Sphingidae). *The Canadian Entomologist, 105*(8), 1041–1052.

Mills, N. J., & Getz, W. M. (1996). Modelling the biological control of insect pests: A review of host-parasitoid models. *Ecological Modelling, 92*(2), 121–143.

Murdock, W. W., Briggs, C. J., & Nisbet, R. M. (2013). *Consumer-resource dynamics*. Princeton University Press.

Nicholson, A. J., & Bailey, V. A. (1935). The balance of animal populations. part I. *Proceedings of the Zoological Society of London, Wiley Online Library, 105*, 551–598.

Reinecke, J. P., Buckner, J. S., & Grugel, S. R. (1980). Life cycle of laboratory-reared tobacco hornworms, *Manduca sexta*, a study of development and behavior, using time-lapse cinematography. *The Biological Bulletin, 158*(1), 129–140.

Self, L. S., Guthrie, F. E., & Hodgson, E. (1964a). Adaptation of tobacco hornworms to the ingestion of nicotine. *Journal of Insect Physiology, 10*(6), 907–914.

Self, L. S., Guthrie, F. E., & Hodgson, E. (1964b). Metabolism of nicotine by tobacco-feeding insects. *Nature, 204*(4955), 300–301.

Snyder, M. J., Walding, J. K., & Feyereisen, R. (1994). Metabolic fate of the allelochemical nicotine in the tobacco hornworm *Manduca sexta*. *Insect Biochemistry and Molecular Biology, 24*(8), 837–846.

Sullivan, D. J. (1987). Insect hyperparasitism. *Annual Review of Entomology, 32*(1), 49–70.

Tougeron, K., & Tena, A. (2019). Hyperparasitoids as new targets in biological control in a global change context. *Biological Control, 130*, 164–171.

Vincent, T. L., & Brown, J. S. (2005). *Evolutionary game theory, natural selection, and Darwinian dynamics*. Cambridge University Press.

Wink, M., & Theile, V. (2002). Alkaloid tolerance in *Manduca sexta* and phylogenetically related sphingids (Lepidoptera: Sphingidae). *Chemoecology, 12*(1), 29–46.

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