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Plant Domestication Alters the Nutritional Content of Guttation Droplets with Multi-trophic Consequences

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Abstract

Domestication often alters plant traits, leading to cascading effects on ecological interactions, particularly in tri-trophic relationships among plants, herbivores, and their natural enemies. While recent studies have investigated the influence of domestication on plant-derived food sources, its effect on guttation—a nutrient-rich exudate produced by many plants—remains unexplored. In this study, we examined the effects of guttation droplets from wild and cultivated highbush blueberries on the fitness (longevity and fecundity) and feeding preferences of three insect species from different trophic guilds: an herbivore (*Drosophila suzukii*), a parasitoid (*Trichopria drosophilae*), and a predator (*Chrysoperla carnea*). Additionally, we analyzed and compared the size and nutritional composition—specifically, total sugar and protein content—of guttation droplets between wild and cultivated blueberry plants. Our results indicated that guttation from wild plants enhanced the longevity of all three insect species, often surpassing that of cultivated plants and diets containing only sugar or sugar plus protein. In choice assays, all three insect species consistently preferred guttation from wild plants over that from cultivated ones. Although the guttation droplets from cultivated plants were larger, those from wild plants contained higher concentrations of sugars (six times more) and proteins (five times more), which likely contributed to the insects' enhanced fitness and preference for wild plant guttation. These findings indicate that domestication has reduced the ecological functionality of guttation in blueberries by potentially influencing tri-trophic interactions. Understanding how domestication affects plant-derived food sources like guttation could have important implications for the conservation of natural enemies in agricultural landscapes.

Keywords Highbush blueberry · *Drosophila suzukii* · *Trichopria drosophilae* · *Chrysoperla carnea* · nutrients

Introduction

Domestication—the process by which humans selectively breed and cultivate plants for agriculture—alters the traits of many crop species. Through this process, wild progenitors are transformed into cultivated crops with desirable traits such as increased yield, improved taste, and enhanced resilience to environmental stress (Gasparini et al. 2021; Krug et al. 2023). However, domestication also induces significant changes in plant physiology, nutritional composition, and defense mechanisms, which can affect interactions between domesticated plants and their surrounding environment, including herbivorous insects (Chen et al. 2015). Research generally shows that domesticated plants have lower defenses, often resulting in increased herbivore performance (Whitehead et al. 2017; Fernandez et al. 2021).

The ecological effects of domestication extend beyond plant-herbivore interactions, influencing higher trophic levels, including the natural enemies of herbivores (Benrey 2023). For instance, domestication can modify volatile emissions from herbivore-damaged plants, which serve as cues that natural enemies use to locate their prey or hosts (Gish et al. 2015; Rowen and Kaplan 2016; Tamiru et al. 2017). Additionally, natural enemies depend on various plant-derived food sources such as nectar, extra-floral nectar, honeydew, pollen and other resources to meet their energy requirements (Wäckers et al. 2005; Wyckhuys et al. 2008; Tena et al. 2016; Heil 2015). Recent studies suggest that domestication can alter the chemical composition of these food sources, potentially leading to significant ecological consequences (Glasser et al. 2023). For example, domestication has modified the chemical composition of nectar and pollen in highbush blueberry (*Vaccinium corymbosum* L.), while reducing pollen chemical diversity in cultivated plants (Egan et al. 2018). These changes may negatively impact pollinator health (Egan et al. 2018) and potentially diminish the fitness of herbivores' natural enemies.

Among plant-derived food sources, guttation—a process through which plants exude droplets of xylem and phloem sap from the edges of their leaves—has traditionally been associated with primary metabolism, particularly in regulating water balance (Singh 2016). However, recent evidence suggests that guttation fluid is often rich in nutrients such as sugars and proteins (Singh 2020), and can play an important role in mediating insect–plant interactions (Singh 2016; Urbaneja-Bernat et al. 2024). For instance, a recent study in highbush blueberry revealed that guttation represents a reliable and nutrient-rich food resource that enhances the fitness of various insect species, including herbivores and their natural enemies (Urbaneja-Bernat et al. 2020a). However, whether domestication has impacted the chemical composition of guttation, and the tri-trophic interactions mediated by guttation, remains unknown.

Highbush blueberry, a perennial crop native to the Northeastern United States and Canada, has a relatively short history of domestication. It was first domesticated just over a century ago in New Jersey (USA), where wild and cultivated blueberries often grow near each other (McCormick 1979). This domestication event led to the development of modern blueberry cultivars with improved berry size and quality, higher plant vigor, and greater yields compared to their wild counterparts (Moore 1965; Wang 2024). Research has shown that the invasive pest *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), commonly known as spotted-wing drosophila, performs better on cultivated than wild blueberry fruits (Rodriguez-Saona et al. 2019). This increased performance correlates with larger fruit size, lower phenolic content, and reduced acidity. Interestingly, *D. suzukii* is more attracted to volatiles emitted from wild fruits (Urbaneja-Bernat et al. 2021). These findings demonstrate that domestication has altered the physical and chemical traits of blueberry fruits, influencing their interactions with a frugivorous insect. While our understanding of how blueberry domestication influences insect–plant interactions is growing, the extent to which domestication affects plant traits beyond fruit characteristics—such as guttation—and the resulting cascading effects on herbivores and their natural enemies remains poorly understood.

Given the importance of plant-derived food sources in sustaining insect communities and mediating ecological interactions, it is crucial to understand how domestication has influenced these resources and their consequences on tri-trophic interactions To address this gap, we hypothesize that domestication has reduced the nutritional content of guttation in blueberries compared to their wild relatives, thereby altering the fitness and preferences of *D. suzukii* and its natural enemies. To test this hypothesis, we conducted a series of laboratory studies with the following specific objectives: (1) to investigate the impact of guttation from wild and cultivated blueberries on the fitness—measured by longevity and fecundity—of insect species representing three feeding guilds: a herbivore (*D. suzukii*), a parasitic wasp [*Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae)], and a predator [*Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)]; (2) to determine the preference of these insects for guttation fluid from wild versus cultivated blueberries through behavioral choice assays; and (3) to analyze and compare the nutritional composition—sugar and protein content—of guttation fluids from wild and cultivated blueberries to identify factors contributing to observed differences in insect fitness and preference. Through this research, we aim to understand how domestication influences the ecological role of plant-derived food sources, such as guttation, in agricultural crops.

Material and Methods

Plant Material

Experiments were conducted using two types of blueberry plants: cultivated highbush blueberry (*V. corymbosum* cv. 'Bluecrop') and wild tetraploid blueberries. Both plant types shared the same ploidy level. Plant material was collected in 2020 and 2021 by propagating cultivated highbush blueberry bushes collected from a field at the P.E. Marucci Center (Chatsworth, Burlington County, New Jersey, USA) and wild blueberry bushes collected from the understory of an adjacent forest dominated by pitch pine (*Pinus rigida* Mill.). The cuttings of the blueberry plants were collected from 4–5 individual bushes within the local population. The propagated plants were used for both 'cultivated' and 'wild' blueberry treatments. Propagation involved taking approximately 10 cm stem cuttings from the mother plants, which were initially planted in 7.6 L (2-gallon) pots filled with a peat and perlite mix (ProMix BX, Quakertown, PA, USA). After rooting, which took about a year, the cuttings were transferred individually to 10.2 cm (4-inch) pots. They were later repotted into 3.8 L (1-gallon) pots containing a 50:50 mix of sand and peat (Premier Pro-Moss, Red Hill, PA, USA; pH = 4–5). All plants were grown in a greenhouse under controlled conditions (20 ± 2°C, 70 ± 10% RH), fertilized twice per week with a granular N:P:K (10:10:10) fertilizer, and irrigated three times per week. At the time of the experiments, the plants were 2-3 years old.

Guttation Sampling and Storage

We manually collected guttation droplets from approximately 40 greenhouse-grown blueberry plants per group (wild and cultivated) between March and June of 2022 and 2023. Groups of approximately five drops were removed from the leaves by gently touching them with a 1 × 1 cm piece of Parafilm® (Sigma-Aldrich, St. Louis, MO, USA), allowing the drops to adhere to the film. The Parafilm® pieces were then stored at −70°C in Petri dishes, labeled by date, until used in experiments. In total, we collected approximately 1,000 guttation droplets across 200 Parafilm® pieces.

Additionally, we estimated the amount of guttation provided to insects in the fitness and preference experiments (see below) to ensure all insects received similar quantities. The volume of each droplet was estimated using the formula $(4/3 \times \pi \times r^3) \times 1/2$, where r is the droplet's radius. Based on this calculation, the total daily amount of guttation offered to insects per Parafilm® piece ($1\ cm^2$)—equivalent to approximately three guttation droplets for cultivated plants and 15 droplets for wild plants—was estimated to be ~0.75 ± 0.005 µl, providing an *ad libitum* diet for all three insect taxa studied. All samples were shipped overnight on dry ice to IRTA, Sustainable Plant Protection Program in Cabrils (Barcelona), Spain, where bioassays were performed and to the Universitat de València, Valencia, Spain for chemical analyses.

Insects

For the experiments, we used insect species from three distinct taxa commonly found in blueberry fields: the herbivore *D. suzukii*; its proovigenic pupal parasitoid, *T. drosophilae* (, which shows promise as a biological control agent for *D. suzukii* in Europe (Wolf et al. 2020); and a generalist predator, the common green lacewing *C. carnea* (), which preys on eggs and first instar larvae of *D. suzukii*, particularly when these stages are not fully enclosed within the fruit (Bonneau et al. 2019; Englert and Herz 2019).

Drosophila suzukii adults were obtained from a laboratory colony, established in 2021 from wild specimens captured in Maresme County (Catalonia, Spain), and maintained at IRTA (Cabrils, Barcelona, Spain). The colony was reared on a standard *Drosophila* artificial diet (Jaramillo et al. 2015) in plastic cups ($350\ cm^3$, 50 mm diameter, 60 mm height) covered with fine mesh secured by a rubber band to allow ventilation, with approximately 25 mL of diet per cup.

Trichopria drosophilae (TRICHOPRIA®) and *C. carnea* (CrisoPack®) were sourced from a commercial supplier (Bioplanet Ibérica S.L, Almeria, Spain). Bottles containing approximately 500 *D. suzukii* pupae parasitized by *T. drosophilae* and frames containing approximately 500 *C. carnea* pupae were placed in separate 25 × 25 × 25 cm methacrylate rearing cages until adult emergence. Emerged adults were provided with wet cotton wool as a water source. All insects were maintained under controlled conditions at 25 ± 1°C, 60 ± 5% RH, and a 16:8 h light-dark photoperiod.

Effects of Guttation on Insect Fitness

We followed the methods described by Urbaneja-Bernat et al. (2020a) to evaluate the effect of guttation on the longevity and fecundity of *D. suzukii*, *T. drosophilae*, and *C. carnea*. To assess longevity, newly emerged adults were isolated in 2 mL glass vials and provided with one of five diets: (1) water droplets, (2) 1 M sugar droplets, (3) 1 M sucrose + protein (5 mg/ml yeast extract) droplets, (4) wild guttation droplets, and (5) cultivated guttation droplets. Since guttation droplets contain sugars and proteins (Urbaneja-Bernat et al. 2020a), artificial sugar and protein diets were included as positive nutritional controls to assess the general capacity of carbohydrate- and protein-rich food sources to support insect performance. These diets were selected based on the concentrations used in Benelli et al. (2017) and Urbaneja-Bernat et al. (2020a). Food sources were provided on Parafilm® pieces and replenished daily. Each Parafilm® piece containing the food treatment was gently folded and inserted at the bottom of the glass vial, ensuring that the food source was accessible to the insects. The insects were able to feed directly from the droplets adhered to the surface of the Parafilm®. Insect survival was monitored daily until death, with 25 replicates per species, sex, and diet.

To assess fecundity, approximately 75 newly-emerged adults (2:1 female:male) of each insect species were placed in transparent cylindrical polypropylene cups (946 mL) and provided with one of five diets *ad libitum* for 24 hours to ensure mating. Following this period, 15 females from each species and dietary group were euthanized and frozen at −20°C (designated as day 1). An additional 30 females per species and diet were individually placed in 25 mL glass vials (designated as day 3-, and 7), where they continued to receive their respective diets *ad libitum*. This setup allowed for egg load assessments at four distinct time points: newly emerged, and at 1-, 3-, and 7-days old. To count mature eggs, frozen females were placed on a microscope slide with a drop of water and gently covered with a coverslip. Light pressure was applied to the thorax using fine entomological pins to expose the ovaries, which were then extruded from the posterior abdomen. The number of mature eggs visible within the ovaries was recorded using a stereomicroscope (Leica MZ16; Leica Microsystems Inc., Buffalo Grove, IL, USA).

Effects of Guttation on Insect Preference

Two-choice bioassays were conducted to test whether newly emerged *D. suzukii*, *T. drosophilae*, and *C. carnea* females preferred guttation droplets from wild or cultivated blueberry plants. The bioassays were performed in a 250-mm diameter glass Petri dish arena (Sigma-Aldrich, St. Louis, MO, USA), where the insects were given the following choices: (1) guttation droplets from wild *vs.* cultivated blueberry plants, (2) wild plant guttation droplets *vs.* water droplets, (3) wild plant guttation droplets *vs.* sucrose + protein solution, (4) cultivated plant guttation droplets *vs.* water droplets, and (5) cultivated plant guttation droplets *vs.* sucrose + protein solution. The droplets were randomly placed on opposite sides of the Petri dish, approximately 12.5 cm apart, on Parafilm® pieces using the same method as in the fitness experiments described above.

A single female *D. suzukii*, *T. drosophilae*, or *C. carnea* was introduced into the center of the arena, which was then covered with the lid. A choice was recorded when the insect fed on one of the droplets for at least 5 seconds. Each individual was used only once, and non-responders (those that did not make a choice) were excluded from data analysis. Each observation lasted 10 minutes per individual, with the experiment replicated 30 times for each choice.

Guttation Size and Nutritional Composition

To measure the diameter of guttation droplets from wild and cultivated plants, droplets were photographed with a digital color camera (Leica DFC500; Leica Microsystems Inc., Buffalo Grove, IL, USA) mounted on a stereomicroscope (Leica MZ16). The diameter of each droplet (*N* = ~500 per droplet type) was measured from the images using ImageJ, a public domain Java-based image processing program (ImageJ: http://rsbweb.nih.gov/ij). Droplet volumes were calculated using the same procedure described above for measuring the daily guttation available to insects.

The concentrations of total sugars and proteins in the guttation samples from both wild and cultivated blueberry plants were measured following methods described by Urbaneja-Bernat et al. (2020a). The overall sugar concentration in the guttation droplets was determined using a modified quantitative anthrone assay (Morse 1947). The same collection method for guttation droplets, as described above, was followed for this assay. To analyze the sugar content, three samples from both cultivated and wild blueberry plants were processed by adding 150 µl of phosphate-buffered saline (8 mM Na_2HPO_4, 2 mM KH_2PO_4, and 150 mM NaCl, pH 7.4) to each sample to dilute the guttation droplets. Further dilutions were made if necessary, depending on the sugar concentrations.

Ten microliters of each diluted sample were placed in a 96-well plate and mixed with 90 µl of anthrone reagent (1.5 mg/ml in 95% sulfuric acid). The plate was stirred for 10 seconds and then incubated for 2 hours at 65°C (hot anthrone test). After incubation, the absorbance at 620 nm was measured using a 96-well plate reader (Tecan Infinite M200Pro; Tecan Austria GmbH, Grödig, Austria). The total sugar concentration was estimated using known concentrations of glucose as standards.

Protein quantification in the same three guttation samples was performed using fluorimetry with a Qubit 3.0 fluorometer (Thermo Fisher, Hercules, CA, USA). One microliter of each sample was analyzed using the Qubit Protein Assay Kit (Thermo Fisher, Hercules, CA, USA) following the manufacturer's instructions.

Statistical Analyses

Kaplan–Meier survival analysis, followed by a log-rank test of equality, was used to assess differences in survival curves across the five diets for each insect species, with separate analyses conducted for each sex. To analyze differences in the number of mature eggs among diets for each insect species at different time points (1-, 3-, and 7-days post-emergence), we employed generalized linear models (GLM) with a Poisson distribution and log link function. A goodness-of-fit test was performed to confirm the suitability of the models. Significant GLM results were followed by Bonferroni pairwise tests. Behavioral bioassay choice test data, and size (diameter and volume) and nutritional composition (sugars and proteins) of plant guttation were analyzed using Student's *t*-tests. All statistical analyses were conducted using IBM SPSS Statistics 23.0 (IBM Inc., Armonk, NY, USA).

Results

Effects of Guttation on Insect Fitness

The longevity of adult males of *D. suzukii*, *T. drosophilidae*, and *C. carnea* was enhanced when fed on wild and cultivated plant guttation or a sugar-only diet compared to diets of sugar plus protein or water only (Fig. 1a,c,e). Additionally, the longevity of females from all three different taxa was the highest when they fed on wild plant guttation, followed by cultivated plant guttation and a sugar-only diet, with the sugar plus protein diet resulting in lower longevity (Fig. 1b,d,f). The water-only diet led to the shortest lifespan.

In general, mated females of *D. suzukii* and *C. carnea* had a higher egg load when given a diet of wild and cultivated plant guttation or the sugar plus protein diet, compared to sugar-only, protein-only, or water-only diets at 1-, 3-, and 7-days post-emergence (Fig. 2a,c). For *T. drosophilidae*, females fed on wild and cultivated plant guttation matured their eggs faster within 1-day post-emergence than when fed on other diets (Fig. 2b). By 3-days after emergence, egg load in all diet treatments were higher than in the water-only group, and no significant differences in egg load were observed among the different food sources when insects were 7 days old (Fig. 2b).

Effects of Guttation on Insect Preference

Choice tests demonstrated that adult *D. suzukii, T. drosophilidae,* and *C. carnea* consistently preferred feeding on wild plant guttation over cultivated plant guttation or any other tested diets (Fig. 3). Moreover, cultivated plant guttation was favored over water by all three taxa, and was preferred over the sugar-plus-protein diet by *T. drosophilidae* (Fig. 3b), though not by *D. suzukii* (Fig. 3a) or *C. carnea* (Fig. 3c).

Guttation Size and Nutritional Composition

Guttation droplets produced by cultivated plants were significantly larger than those from wild plants, both in diameter ($t = 10.69$, df = 357, $P < 0.001$) and volume ($t = 10.69$, df = 357, $P < 0.001$) (Fig. 4a,b).

Chemical analyses showed that total sugar concentration in guttation from wild plants was approximately six times higher than in cultivated plants ($t = 3.32$, df = 4, $P = 0.029$) (Fig. 4c). Similarly, protein concentrations were estimated to be five times higher in guttation droplets from wild plants compared to those from cultivated plants ($t = 4.38$, df = 4, $P = 0.012$) (Fig. 4d).

Discussion

Domestication is widely recognized as a key driver of changes in plant traits (Gasparini et al. 2021; Krug et al. 2023). It is also well-established that domestication can alter plant chemistry, thereby influencing interactions among plants, herbivores, and their natural enemies (Chen et al. 2015; Benrey 2023). Our study provides new evidence that insects from three feeding guilds—the herbivore *D. suzukii*, the parasitoid *T. drosophilae*, and the predator *C. carnea*—exhibited improved fitness, measured by greater adult longevity (Fig. 1), when fed guttation droplets from wild blueberry plants compared to those from cultivated varieties. Furthermore, when given a choice, all three species preferred to feed on guttation from wild plants. This fitness–preference relationship was associated with the higher sugar and protein content found in the guttation of wild blueberries. These findings align with broader trends observed in other plant-derived food sources, such as nectar and pollen, where domesticated crops often exhibit altered nutritional profiles—sometimes to the detriment of beneficial insects like pollinators (Egan et al. 2018).

Guttation from both wild and cultivated blueberry plants enhanced the longevity of *D. suzukii*, *T. drosophilae*, and *C. carnea* adults, performing comparably to a sugar-only diet and surpassing the benefits of a sugar-plus-protein diet. These results suggest that while sugar is essential for insect survival, the artificial protein source (yeast extract) was less beneficial than the natural protein present in guttation droplets. Furthermore, the fecundity of all three insect species increased when fed guttation or a sugar-plus-protein diet, indicating that protein is essential for egg production. Although artificial diets served as positive nutritional controls and were not designed to replicate the exact composition of guttation, the fact that wild guttation performed as well as—or in some cases better than—these controls highlights its value as a naturally occurring, high-quality food source. These findings build upon those of Urbaneja-Bernat et al. (2020a), who reported that *D. suzukii*, the parasitoid *Aphidius ervi* Haliday, and the generalist predator *Chrysoperla rufilabris* (Burmeister) exhibited enhanced fitness when fed guttation from cultivated blueberries. As guttation is secreted from leaves throughout the growing season (Urbaneja-Bernat et al. 2020a), it may provide a more consistent food resource for insects in natural habitats than pollen or floral and extrafloral nectar.

Natural habitats surrounding agricultural farms often serve as refuges and overwintering sites for insects, offering protection from both biotic and abiotic stressors, such as pesticide exposure (Rusch et al. 2010; Clem and Harmon-Threatt 2021). These habitats also contain wild plants that can provide shelter and alternative food sources for insects (Tscharntke et al. 2007; Gardiner et al. 2009; Chaplin-Kramer et al. 2013; Redlich et al. 2018; Feit et al. 2021). For instance, non-crop habitats near farms are a source of *D. suzukii* adults, which migrate into crops during the growing season (Urbaneja-Bernat et al. 2020b; Buck et al. 2023). They also provide essential food sources for beneficial insects, such as pollen and extrafloral and floral nectar (Wäckers et al. 2007). This study provides further evidence that these natural habitats may also supply guttation from wild plants, such as wild blueberries, which benefit insects.

Interestingly, our study demonstrates that guttation benefits both herbivores and their natural enemies. Wild blueberries have been shown to serve as reservoirs for *D. suzukii* during fruit ripening, acting as potential sources of infestation for nearby highbush blueberry fields (Urbaneja-Bernat et al. 2020b). Additionally, Urbaneja-Bernat et al. (2021) reported that blueberry domestication reduces fruit volatile emissions, which in turn diminishes *D. suzukii* attraction to cultivated fruits relative to wild ones. Despite these lower volatile emissions, domesticated blueberries are more susceptible to *D. suzukii* infestation (Rodriguez-Saona et al. 2019). The present study extends these findings by showing that domestication has also shaped tri-trophic interactions. Natural enemies of herbivores, such as those targeting *D. suzukii*, benefit from feeding on guttation from wild blueberries. In fact, a previous field study (Urbaneja-Bernat et al. 2020a) found that plants producing guttation droplets attracted significantly more parasitoids and predators than herbivores, suggesting that the net ecological effect of guttation may favor biological control. This suggests that non-crop habitats containing wild blueberries may enhance biological control and help mitigate pest pressure in adjacent cultivated fields.

Our study demonstrates that domestication influences both the quantity (i.e., size) and quality (i.e., nutrient content) of plant guttation. Cultivated blueberry plants produce larger guttation droplets than their wild counterparts, potentially providing greater resource availability for insects in the field. However, this increase in droplet size is accompanied by a reduction in nutritional quality, with guttation from cultivated plants exhibiting lower content of sugars and proteins compared to those from wild plants. This suggests that insects may need to spend more time feeding on guttation from cultivated plants to obtain the same amount of nutrients as they would from wild plants. A similar domestication syndrome was reported by Rodriguez-Saona et al. (2019), where cultivated blueberry bushes produce significantly larger fruits compared to wild bushes, resulting in lower sugar and phenolic content per unit of weight.

The sugar concentration in guttation from wild blueberry plants (Fig. 4c) falls within the lower range of values reported for floral nectar, which typically contains 10–50% sugar by weight, depending on plant species and ecological context (Wäckers et al. 2007; Nicolson and Thornburg 2007). Sugars serve as rapidly metabolized energy sources and are essential for daily activity and metabolic maintenance in insects (Wäckers et al. 2005). They are especially critical for flight in insects with high wing-beat frequencies, such as members of the Diptera and Hymenoptera (Behmer 2009). These insects rely on the rapid oxidation of sugars to power their flight muscles (Wäckers et al. 2008), which likely explains the greater longevity and strong feeding preference exhibited by *D. suzukii*, *T. drosophilae*, and *C. carnea* when offered guttation from wild plants, which had higher sugar content than cultivated counterparts. This reliance on sugar-rich food highlights the ecological importance of wild plants in sustaining insect populations and supporting tri-trophic interactions in agroecosystems.

We also found that protein content was significantly higher in guttation droplets from wild blueberry plants compared to those from cultivated ones (Fig. 4d). Protein levels in wild guttation exceeded those typically found in floral nectar, which often contains negligible or trace amounts of amino acids (Heil 2011; Nepi et al. 2018). Although protein-rich diets are known to support insect growth and reproduction under certain conditions (Maklakov et al. 2008; Behmer 2009; Benelli et al. 2017; Espinosa et al. 2021), the elevated protein content in wild guttation did not lead to increased egg production in our study. Egg load measurements revealed no significant differences between insects fed on wild or cultivated guttation and those provided with an artificial sugar-plus-protein diet. This suggests that, although wild guttation contained more protein, both guttation types likely provided sufficient protein to support reproductive output under laboratory conditions. Nevertheless, the higher protein content in wild guttation may offer advantages in more nutritionally variable or stressful field environments.

In conclusion, our study demonstrates that blueberry domestication has notable ecological consequences by altering the chemical composition of plant guttation. Guttation from wild blueberries—rich in both sugars and proteins—more effectively supports the longevity and feeding preference of an herbivore, a predator, and a parasitoid compared to guttation from cultivated plants and artificial diets. These findings underscore the complex effects of domestication: while reduced volatile emissions in cultivated blueberries may decrease pest attraction, increased susceptibility to *D. suzukii* and the reduced nutritional quality of guttation for natural enemies could present challenges for pest management. In contrast, wild blueberries in non-crop habitats may help sustain populations of beneficial insects, potentially enhancing biological control in adjacent fields. However, because the more nutritious guttation from wild plants also benefits *D. suzukii*, further research is needed to understand how domestication-driven changes in guttation influence the balance between herbivores and their natural enemies, and ultimately, the efficacy of biological control in agroecosystems.

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Declarations

Ethics approval and consent to participate Not applicable.

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Figure Captions

Figure 1. Survival curves for the spotted-wing drosophila *Drosophila suzukii* (a,b), *Trichopria drosophilae* (c,d), and *Chrysoperla carnea* (e,f) females and males fed on five different diets: wild guttation droplets, cultivated guttation droplets, sugar only, sugar plus protein, and water. Different letters indicate significant differences among diet treatments according to log-rank test of equality. *N* = 25 per species, sex, and diet.

Figure 2. Egg load (mean number of eggs ± SE) of *Drosophila suzukii* (a), *Trichopria drosophilae* (b), and *Chrysoperla carnea* (c) fed on five different diets: wild guttation droplets, cultivated guttation droplets, sugar only, sugar plus protein, and water. Counts were taken 1-, 3-, and 7-days after adult emergence. Different letters indicate significant differences among diet treatments according to the Bonferroni pairwise tests. *N* = 15 females from each species and dietary group.

Figure 3. Percent response (mean ± SE) of *Drosophila suzukii*, *Trichopria drosophilae*, and *Chrysoperla carnea* females to four different diets: wild guttation droplets, cultivated guttation droplets, sugar plus protein, and water. Asterisks indicate significant differences between diet choices based on *t*-test. Each bar represents the mean of 30 replicates, with one female from each insect species tested per replicate.

Figure 4. Mean (± SE) for the diameter (a), volume (b), sugar content (c), and protein content (d) of guttation droplets from wild and cultivated plants. Asterisks indicate significant differences between wild and cultivated plants based on *t*-tests. For (a) and (b), *N* = ~500 per droplet type; for (c) and (d), *N* = 3 per droplet type.

Fig 1.

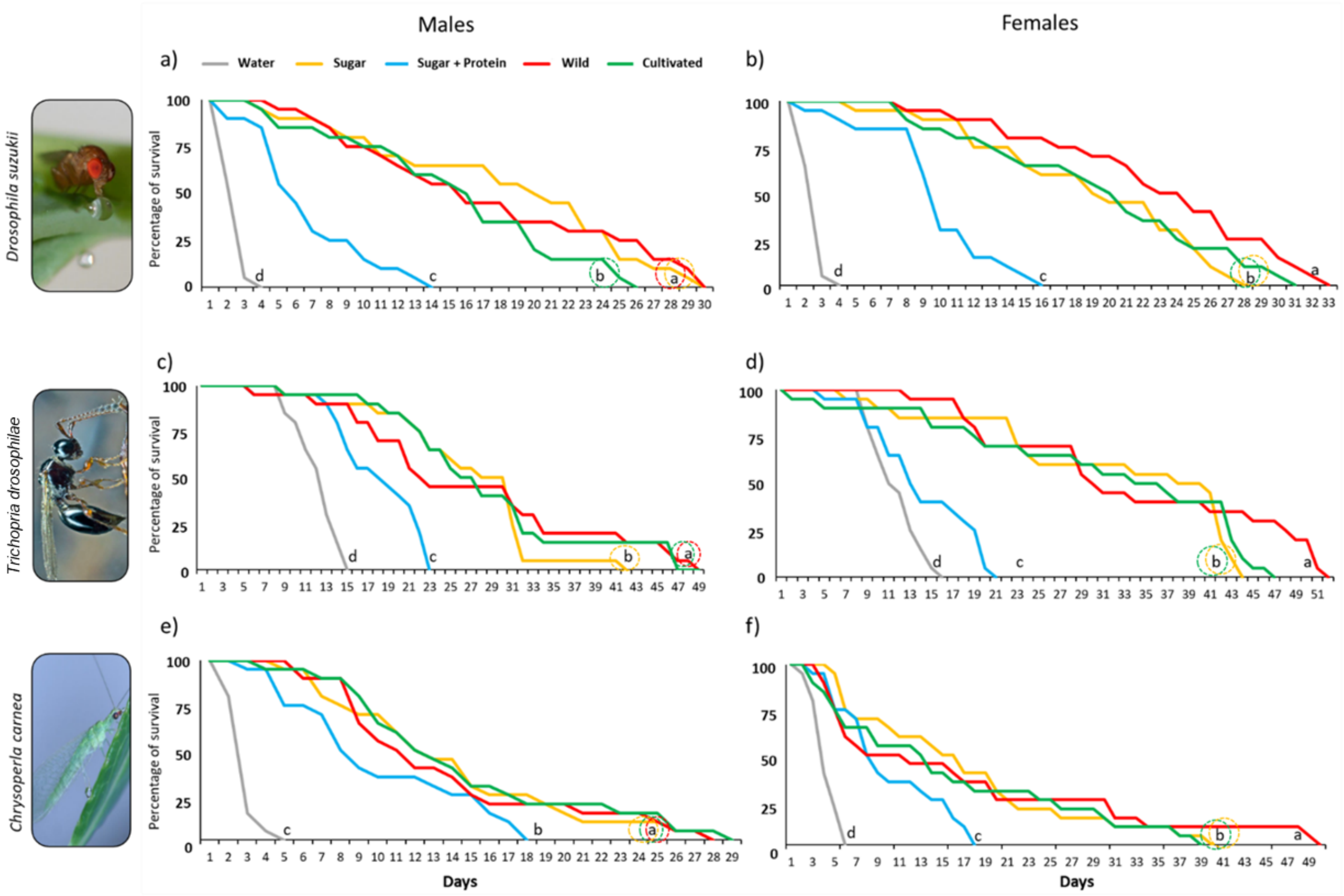

Males
Females
a)
b)
c)
d)
e)
f)
Water
Sugar
Sugar + Protein
Wild
Cultivated
Drosophila suzukii
Trichopria drosophilae
Chrysoperla carnea
Percentage of survival
100
75
50
25
0
a
b
c
d
Days

Fig 2.

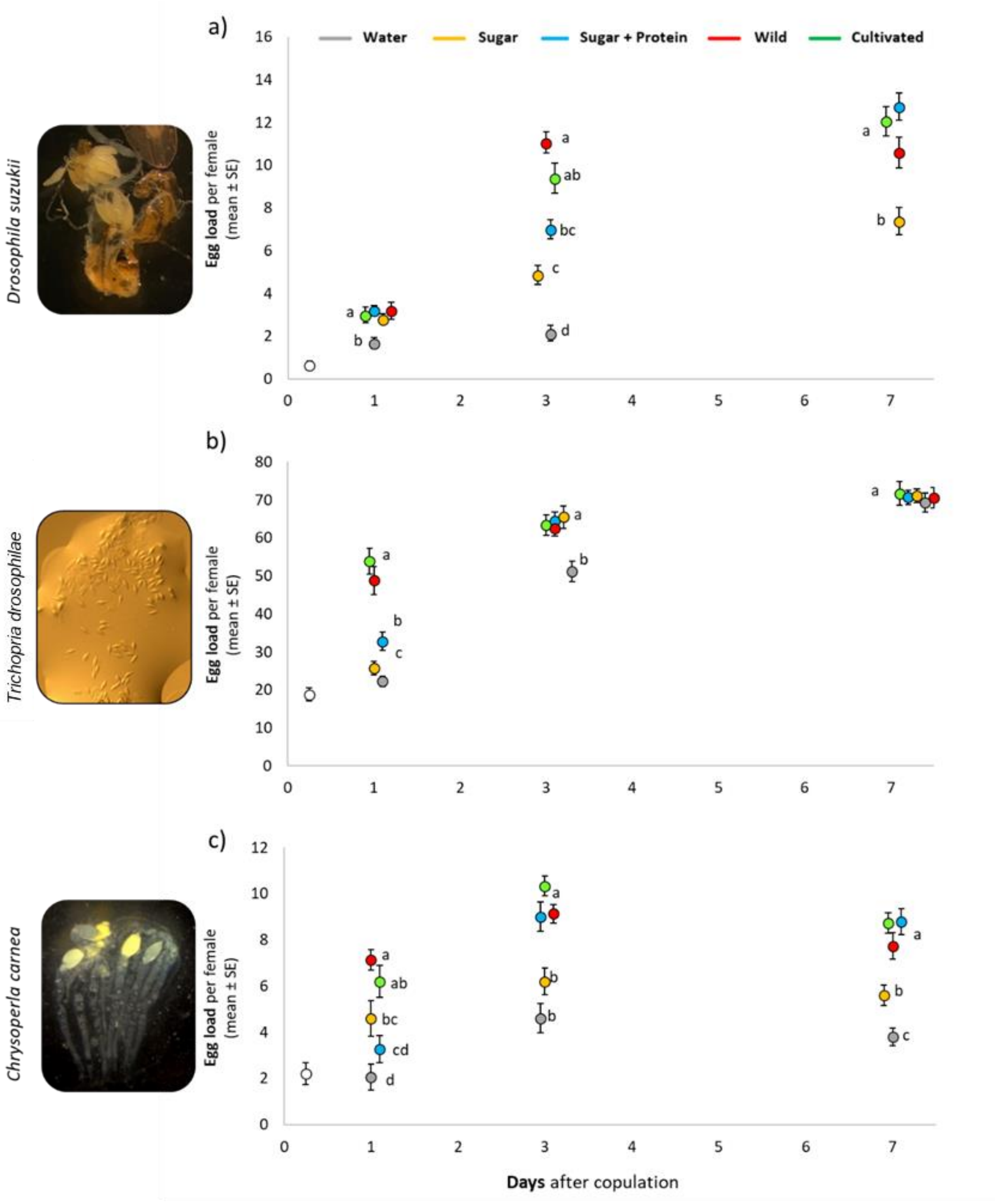

a)
Water
Sugar
Sugar + Protein
Wild
Cultivated
Drosophila suzukii
Egg load per female (mean ± SE)
16
14
12
10
8
6
4
2
0
a
b
a
ab
bc
c
d
a
b
0
1
2
3
4
5
6
7
b)
Trichopria drosophilae
Egg load per female (mean ± SE)
80
70
60
50
40
30
20
10
0
a
b
c
a
b
a
0
1
2
3
4
5
6
7
c)
Chrysoperla carnea
Egg load per female (mean ± SE)
12
10
8
6
4
2
0
a
ab
bc
cd
d
a
b
b
a
b
c
0
1
2
3
4
5
6
7
Days after copulation

Fig 3.

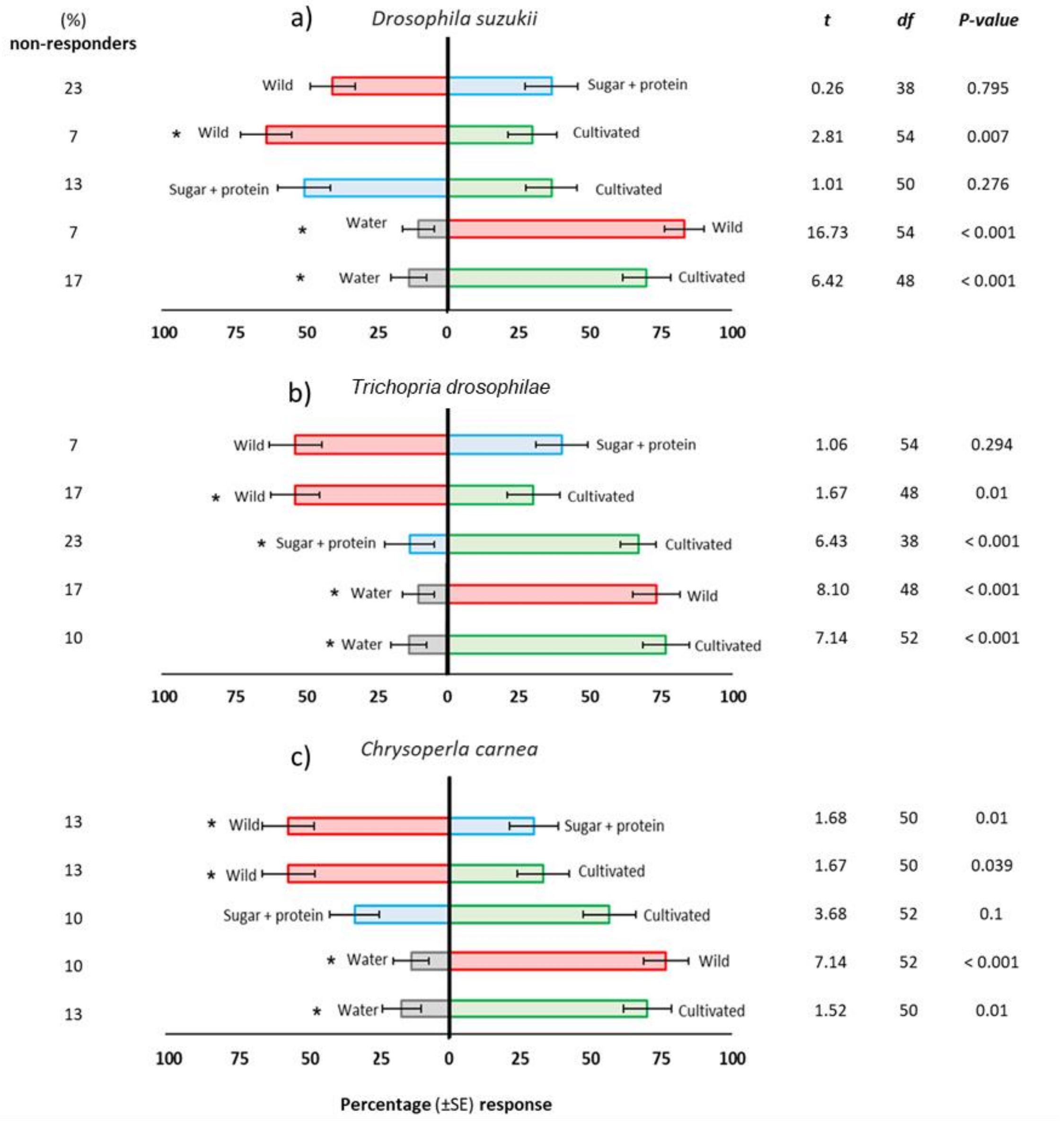
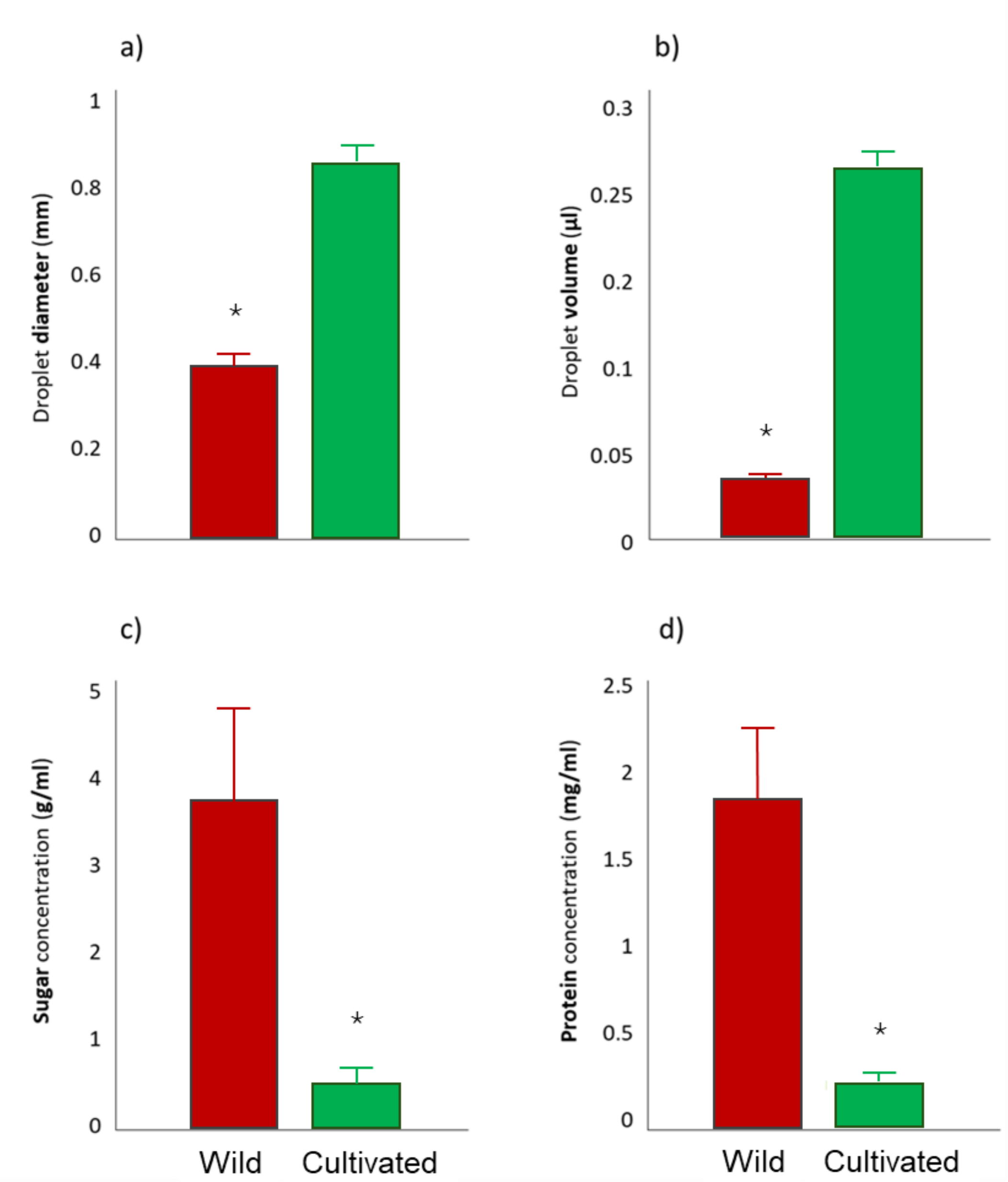

(%)
non-responders
a)
Drosophila suzukii
t
df
P-value
23
Wild
Sugar + protein
0.26
38
0.795
7
* Wild
Cultivated
2.81
54
0.007
13
Sugar + protein
Cultivated
1.01
50
0.276
7
* Water
Wild
16.73
54
< 0.001
17
* Water
Cultivated
6.42
48
< 0.001
100 75 50 25 0 25 50 75 100
b)
Trichopria drosophilae
7
Wild
Sugar + protein
1.06
54
0.294
17
* Wild
Cultivated
1.67
48
0.01
23
* Sugar + protein
Cultivated
6.43
38
< 0.001
17
* Water
Wild
8.10
48
< 0.001
10
* Water
Cultivated
7.14
52
< 0.001
100 75 50 25 0 25 50 75 100
c)
Chrysoperla carnea
13
* Wild
Sugar + protein
1.68
50
0.01
13
* Wild
Cultivated
1.67
50
0.039
10
Sugar + protein
Cultivated
3.68
52
0.1
10
* Water
Wild
7.14
52
< 0.001
13
* Water
Cultivated
1.52
50
0.01
100 75 50 25 0 25 50 75 100
Percentage (±SE) response

Fig 4.


a)
Droplet diameter (mm)
1
0.8
0.6
0.4
0.2
0
*
b)
Droplet volume (µl)
0.3
0.25
0.2
0.1
0.05
0
*
c)
Sugar concentration (g/ml)
5
4
3
2
1
0
*
Wild
Cultivated
d)
Protein concentration (mg/ml)
2.5
2
1.5
1
0.5
0
*
Wild
Cultivated