https://doi.org/10.35662/unine-thesis-2905

UNIVERSITÉ DE NEUCHÂTEL

E-V-L Laboratory of evolutionary entomology

Consequences of the domestication of chili pepper on multitrophic interactions

A dissertation submitted to the University of Neuchâtel

for the degree of Doctor in Natural Sciences presented by

Yosra Chabaane

Thesis director

Prof. Betty Benrey, University of Neuchâtel, Switzerland

Thesis committee members

Prof. Pilar Junier, University of Neuchâtel, Switzerland

Dr. Carla Cristina Marques Arce, University of Neuchâtel, Switzerland

Dr. Tim Haye, CABI, Switzerland

Defended on August 6th, 2021

UNIVERSITÉ DE
NEUCHÂTEL

Faculté des Sciences
Secrétariat-décanat de Faculté
Rue Emile-Argand 11
2000 Neuchâtel – Suisse
Tél : + 41 (0)32 718 21 00
E-mail : secretariat.sciences@unine.ch

IMPRIMATUR POUR THESE DE DOCTORAT

La Faculté des sciences de l'Université de Neuchâtel autorise l'impression de la présente thèse soutenue par

Madame Yosra CHABAANE

Titre:

"Consequences of the domestication of chili pepper on multitrophic interactions"

sur le rapport des membres du jury composé comme suit:

- Prof. tit. Betty Benrey, directrice de thèse, Université de Neuchâtel, Suisse
- Prof. Pilar Junier, Université de Neuchâtel, Suisse
- Dre Carla Cristina Marques Arce, Université de Neuchâtel, Suisse
- Dr Tim Haye, CABI, Delémont, Suisse

Neuchâtel, le 19 août 2021

Le Doyen, Prof. A. Bangerter

Content

1. General introduction

Securing food provision for the world population involves geopolitical, economic, societal, and agricultural actions. The second goal of the United Nations' Sustainable Development Goals (SDGs) aims to end hunger, decrease food losses and waste, improve nutrition, and promote sustainable agriculture by 2030 (Bebbington and Unerman 2018). Currently, there is a trend to encourage farming models that decrease chemical inputs and increase biodiversity. In this context, plant domestication plays a central role in enhancing food security. Indeed, it is considered a source of agricultural diversification and a pathway for improved nutrition and income, especially for smallholders (Sassi et al. 2018).

1.1. Plant domestication and effects on multitrophic interactions

Plant domestication is defined as the genetic selection process to adapt wild plants to cultivation, whether consciously or unconsciously done by humans (Gepts and Papa 2001). Many plant traits were changed during this process (e.g., increased fruit size and yield, changed color, decreased toxicity) known as domestication syndrome (Hammer 1984). The most common trait altered by domestication was secondary plant metabolites (e.g., Reduction of toxic compounds), followed by morphological changes (e.g., Changes of vegetative parts or fruit size) (Meyer et al. 2012). These altered traits were reported to increase plant susceptibility to herbivores (Benrey et al. 1998; Gols et al. 2008; Idris and Grafius 1996). However, these patterns are not ubiquitous (Chen et al. 2015a; Chen et al. 2015b; Whitehead et al. 2017). For example, Turcotte et al. (2014) compared the performance of the leaf chewing herbivore beet armyworm (*Spodoptera exigua*) and the phloem-feeding green peach aphid (*Myzus persicae*) on 29 crop species and their wild ancestors. They found that although domestication mainly reduced plant defenses, it does not always cause an increase in herbivore performance. It suggests that results are crop dependant. However, this study focused on generalist herbivores, which might be more affected by plant defenses than specialists pests (Ali and Agrawal 2012). Therefore, the effects of altered traits by domestication are expected to be different on specialists than generalist insects. Indeed, Gaillard et al. (2018) showed that generalist insects are more vulnerable than specialist insects when feeding on teosinte (ancestor of Maize). The consequences of plant domestication might differ on insects feeding on different plant tissue (Shlichta et al. 2018). In a recent study, Jaccard et al. (2021) showed that cucurbitacins were detected in significant quantities in cotyledons and roots of Squash varieties (*Cucurbita* spp.). However, leaves have a high density of trichomes density but a minimal amount of cucurbitacins. This tradeoff in plant tissue defenses did not affect the performance of the leaf herbivore (*Spodoptera latifascia*) and the root beetle (*Diabrotica balteata*).

Plant domestication can also influence the third trophic level through changing the indirect plant defenses (e.g., volatile organic compounds) (Chen et al. 2015a; de Lange et al. 2016; Gols et al. 2008; Turlings and Benrey 1998). For example, parasitoid wasps can benefit from the volatiles emitted from herbivore-damaged plants and use them as cues to find their host (Turlings et al. 1990; Vet and Dicke 1992). In addition, secondary metabolites can slow down herbivore development and thus, increase their time of exposure to natural enemies (Benrey and Denno 1997; Price et al. 1980). Alternatively, some herbivores can sequester and store plant toxins in their bodies, making them unpalatable or toxic for their natural enemies (Opitz and Müller 2009; Rowell-Rahier and Pasteels 1992).

For chili pepper, nonpathogenic viruses are prevalent and maintained in a persistent lifestyle in plants. It was suggested that the ancestor, hot pepper endonavirus CFEV 1, might have evolved as a new endonavirus for Bell pepper called BPEV in *C.annum* non-pungent varieties (Safari and Roossinck 2018). Whitefly-infested viruses were also reported to attack chili crops, causing severe damage (De Barro et al. 2008; Maruthi et al. 2007). Interestingly, most Chiltepin plants might be asymptomatic thanks to their genetic diversity and the reduced plant density (Fraile et al. 2017). However, the comparison in plant response between wild and cultivated chili pepper to virus infection remains unstudied.

In the case of chili pepper, the consequences of plant domestication are poorly investigated despite using this crop worldwide. Only a handful study found that caterpillars of *Manduca sexta* (the tobacco hornworm) performed equally well on the domesticated and wild chili leaves, but they were less parasitized when feeding on wild plants, Chiltepin (Garvey et al. 2020). However, this study did not look at the defensive traits responsible for these plant-insect interactions. Therefore, my thesis explored the altered plant traits on fruits, flowers, and leaves and investigated multiple interactions to have robust data regarding the consequences of chili domestication.

1.2. Domestication of chili pepper

Chili pepper offers an interesting study model in which domestication aimed to decrease and increase their main secondary metabolites, called capsaicinoids, synthesized and accumulated in fruits (Pandhair and Sharma 2008; Pickersgill 2016). Varieties were selected along a gradient of pungency levels from 0 to 2,200 000 Scoville Heat Unit (SHU), called Scoville chart (Scoville 1912). This scale was developed by the pharmacologist Wilbur Lincoln Scoville in 1912 to characterize chili varieties according to their spiciness. Each unit in this scale corresponds to the number of dilutions made to obtain a non-pungent solution. In addition to capsaicinoids level, the domestication syndrome for chili pepper included also increase in fruit size, changes in fruit color and thickness, changes in the fruit position from upright to pendant hidden by the foliage where the fruit is more protected from bird dispersal and remains attached to the plant when fully ripe (Ladizinsky 2012; Pickersgill 2016). Domesticated peppers also have larger leaves, flowers, and seeds than wild peppers (Pickersgill 2007). The only consistent trait-distinguishing wild from domesticated chilies is the rapid uniform germination in chili varieties necessary for producing higher yields (Pickersgill 2007). Moreover, wild chili grows as a perennial shrub (up to 8 m of height) under nurse trees living for more than a decade (Kraft et al. 2013; Perramond 2005), whereas domesticated varieties are mainly cultivated as annual plants of (30 cm to 2 m height) in greenhouses or open field (Liu et al. 2021; Maaouia-Houimli et al. 2011).

1.3. Origin and main domesticated species

It was suggested that the genus *Capsicum* is most likely originated from the Andean mountains, then reached lowland regions in the Americas (Walsh and Hoot 2001b). Although chili pepper was not an essential crop before the European conquest, its domestication dates to 10 000 years ago (Aguilar-Meléndez et al. 2009). This genus includes around 35 species (Carrizo García et al. 2013), from which five were domesticated in different parts of Mexico, Central and South America (Pickersgill 1997) (Figure 1). The white-flowered group, known as the *C. annum* complex, includes the most domesticated chili species worldwide (*C. annum*, *C. chinense*, and *C. frutescens*) that can be crossed with each other (Pereira-Dias et al. 2019). *C. baccatum* is another cultivated species with white flowers that have a yellow spot at the base. Finally, the purple-flowered species includes the domesticated species *C. pubescens*. Each species is derived from a different wild ancestor (Bosland et al. 2012). The wild Chiltepin (*C. annuum var. glabriusculum*) is most likely the ancestor of *C. annuum* and was domesticated in Mexico (Kraft et al. 2014). *Capsicum baccatum* and *C. pubescens* are mainly cultivated in South America and derived from their wild relatives, respectively *C. tovarii* Eshbaugh, Smith et Nickrent, and *C. cardenasii* Heiser et Smith (Pereira-Dias et al. 2019; Tong and Bosland 2004). However, the wild ancestors and evolutionary history of *C. frutescens* and *C. chinense* remain unclear (Carvalho et al. 2014; Walsh and Hoot 2001b). The geographical distribution of *C. frutescens* is expanded from the lowlands of southeastern Brazil to the Antilles in the Caribbean (Carvalho et al. 2014), and *C. chinense* was diversified in the Amazon region, but some varieties are originally from Mexico (e.g., Habanero) and Caribbean (e.g., Scotch Bonnet) (Antonious et al. 2009; Eshbaugh 1993; McGlashan et al. 1993).

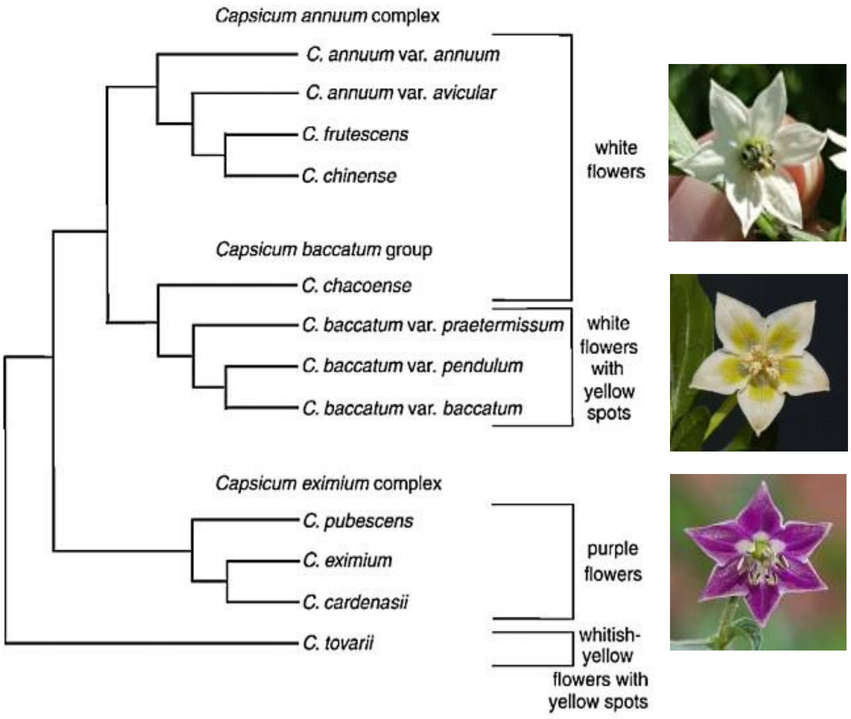


Fig. 1 Phylogenetic relationship between domesticated species of the genus *Capsicum*. The classification is based on flower color (Walsh and Hoot 2001a).

1.4. Purpose of domestication

Chili pepper is among the most cultivated plants globally (Andrews 1995; FAOSTAT 2020) and has become a crucial ingredient in many international cuisines. In their area of origin, chili pepper has a multiple use in addition to domestication for consumption. For example, it was known to treat different diseases such as respiratory problems, earache, and stomachache since the Mayan civilization in Mesoamerica (Cichewicz and Thorpe 1996; Pickersgill 2016). In the Aztec culture, children were punished with chili smoke (Figure 2). Thanks to its antibacterial properties, chili pepper was also used for food preservation before refrigerators (Omolo et al. 2014; Perry and Flannery 2007). In Bolivia, it was reported that *C. baccatum* species had a wide range of applications, such as a natural coloring agent, highly pungent self-defense sprays, and repellent extracts against animals and insects (Bosland 1996). The ornamental use of chili pepper started with the Indian cultures in Mesoamerica as part of their religious ceremonies. Then, it was transferred and appreciated by Europeans when introduced in the 15th century (Stommel and Bosland 2006).

Outside their area of origin, chili pepper has also become popular and even a symbol of identity for certain regions worldwide. For example, the chili variety "*le piment d'Espelette*" selected in Espelette at the French-Spanish border was used to create patches for muscular pains and as a spice for the traditional sausages to replace black pepper, which was expensive and scarce (Boulanger-Fassier 2013). In Tunisia, chili pepper arrived with the emigration of Muslims and Jews from Andalusia (Spain) to North Africa in the 17th century (Atigue 2019; Ben Achour 2020; Diallo 2020). Since then, this ingredient has become an essential condiment of Tunisian cuisine and neighboring countries, especially with their famous chili paste called "*Harissa*" (Othmani 2020). In some African and Asian countries, farmers plant chili pepper as a barrier to repel Elephants and avoid losing their crops (e.g., Maize) (Chang'a et al. 2016; Hedges and Gunaryadi 2010). This practice aimed to reduce the human-elephant conflict and save the life of these mammals.

Fig. 2 Chili pepper was used for child punishment by Aztec parents. The dad holds his son over smoking chilies (**left**), and the mum threatens her daughter with the same treatment (**right**). Image from the Codex Mendoza (original in the Bodleian Library, Oxford).

1.5. Study system

My thesis concerns the consequences of chili pepper domestication on multitrophic interactions. I used the generalist herbivore *S. latifascia* and its ectoparasitoid *E. platyhypenae* (**chapter one**), studied the primary attackers of chili pepper in their natural habitat focusing on whiteflies and their associated virus (**chapter two**). Finally, I examined the feeding and oviposition behavior of the specialist pest, pepper weevil (*A. eugenii*) (**chapter three**). We used several organisms to deeply investigate how altered traits on different plant tissues may affect multitrophic interactions.

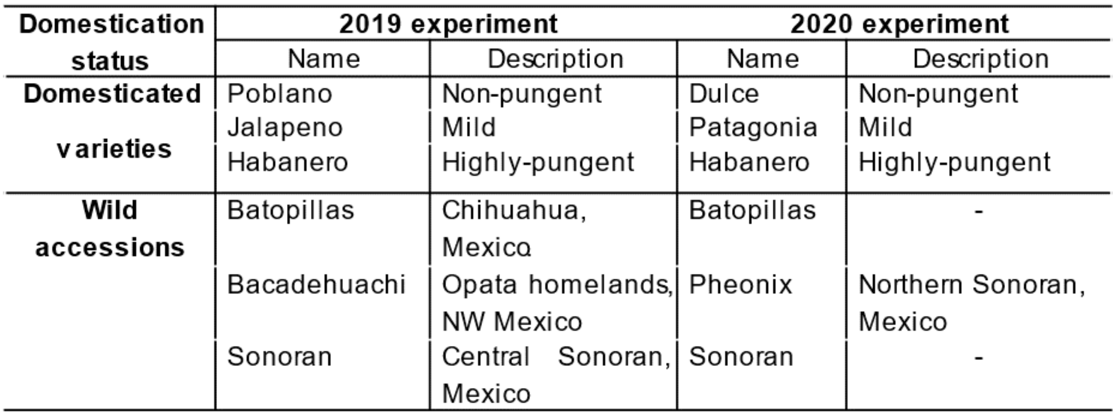
1.5.1. Chili pepper

In this thesis, we used wild accessions and cultivated varieties selected for consumption and as ornamental. We focused our study mainly on *C. annum* species domesticated in Mexico, where we conducted our fieldwork. Indeed, centers of domestication where crops coexist with their wild relatives are key field sites to investigate how local biotic and abiotic environments and human preferences influenced crop domestication and diversification. Thus, native insect and pathogen species associated with wild ancestors have possibly contributed to the selection of plant phenotypes (Chen et al. 2017).

For **chapter one**, three chili varieties were selected based on their known pungency level: non-pungent variety Padron (*C. annuum*), mild variety Cayenne (*C. annuum*), and highly pungent variety, Habanero (*C. chinense*). These varieties are originally from Latin America (Muñoz-Ramírez et al. 2018), except for Padron, selected in Galicia, Spain (Katz 2009).

Chapter two used a combination of three wild accessions and three domesticated varieties for two successive seasons 2019 and 2020, as summarized in Table. 1 They are all *Capsicum annuum* species except for the Habanero variety (*C. chinense*), known to be more pungent than the wild Chiltepin (*C. annuum var. glabriusculum*) according to the Scoville scale (Scoville 1912). This variety was selected in the Yucatan Peninsula in Mexico (Muñoz-Ramírez et al. 2018) and widely used in the state of Oaxaca (Marina Clemente et al. 2020).

Table 1. Summary of domesticated varieties and wild accessions used for the common garden experiments during 2019 and 2020 seasons in Puerto Escondido (Oaxaca, Mexico).



Domestication status	2019 experiment		2020 experiment	
	Name	Description	Name	Description
Domesticated varieties	Poblano	Non-pungent	Dulce	Non-pungent
	Jalapeno	Mild	Patagonia	Mild
	Habanero	Highly-pungent	Habanero	Highly-pungent
Wild accessions	Batopillas	Chihuahua, Mexico	Batopillas	-
	Bacadehuachi	Opata homelands, NW Mexico	Pheonix	Northern Sonoran, Mexico
	Sonoran	Central Sonoran, Mexico	Sonoran	-

In **chapter three**, we used not only domesticated varieties selected for consumption (Scotch Bonnet, Jalapeño, and Peperoncino) but also five ornamental pepper varieties (Pops Yellow, Black Pearl, Sedona Sun, Chilli Chilli, and Salsa Deep) in addition to one wild accession (Birds Eye Pepper). All these plants, except for Peperoncino, were extensively grown in Florida, where the pepper weevil is a big pest on chili peppers in open fields and greenhouses (Qureshi and Kostyk 2020; Wu et al. 2019). They all belong to *C. annum* species except for Scotch Bonnet, *C. chinense,* known to be more pungent than the wild Chiltepin (*Capsicum annuum var. glabriusculum*) according to the Scoville scale (Scoville 1912).

1.5.2. Generalist insects

- ***Spodoptera latifascia* and its ectoparasitoid (*Euplectrus platyhypenae*)**

Spodoptera latifascia (Lepidoptera: Noctuidae), commonly known as the Velvet armyworm, occurs naturally throughout Mexico and Central America (King and Saunders 1984; Zagatti et al. 1995). It is a polyphagous insect whose host range includes several crops such as potato, cotton, soybeans, Maize, and beans (Cuny et al. 2018; Habib et al. 1982). Larvae have been frequently found feeding on leaves of chili plants in Mexico (Traine et al. 2020), and it was also observed feeding on fruits (Chabaane, personal observation). In turn, this herbivore is attacked by the gregarious koinobiont ectoparasitoid *Euplectrus platyhypenae* (Hymenoptera: Eulophidae), originally also from Mexico and parasitizes the third and fourth instars of Noctuid and Geometrid caterpillars (Muniappan et al. 2004; Murúa and Virla 2004; Swezey 1924). Before oviposition, female wasps inject venom on the dorsum of the caterpillar to inhibit molting without killing their host. One female lays up to 20 eggs that develop on the dorsal segments of the host's body, feeding on its haemolymph (Coudron et al. 1990; Nakamatsu and Tanaka 2003) (Figure 3 A). Before pupation, the parasitoid larvae (Figure 3 B) use their saliva to kill the host and move underneath the cadaver to pupate (Nakamatsu and Tanaka 2004) (Figure 3 C). Approximately, one-week later, adults emerge (Nakamatsu and Tanaka 2003) (Figure 3 D).

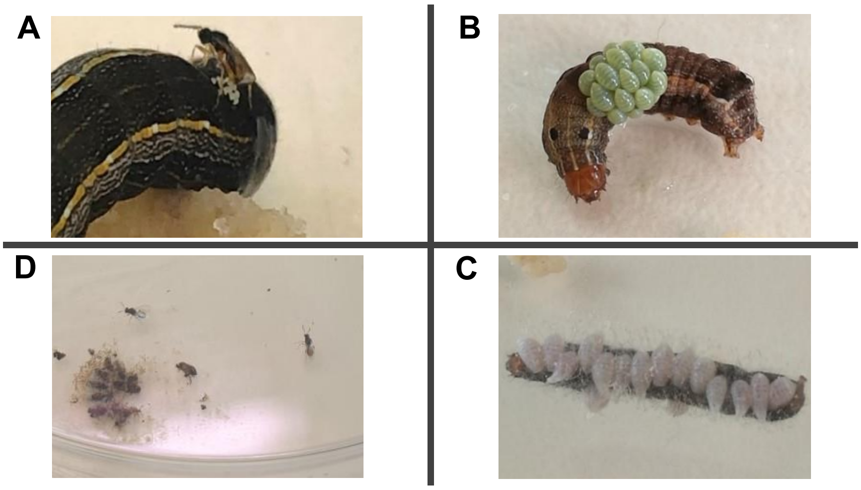


Fig. 3 Life cycle of *Euplecterus platyhypenae* on its host, *Spodoptera latifascia*. A) Oviposition, female parasitoid laying eggs on a fourth instar larva of *Spodoptera latifascia*. B)

Parasitoid larva on the dorsum of their host. C) Parasitoid pupa under the caterpillar body. D) Emergence of adult parasitoid from the dried pupa. All images © Y. Chabaane.

- **Whiteflies and their associated vector**

The fieldwork revealed that whiteflies associated with their vectors were the main attackers of chili plants (**chapter two**). One of the most destructive pests of chili peppers is the sweet potato whitefly (SPW), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Ballina-Gomez et al. 2013; Nasruddin et al. 2020). It is a sapsucker phytophagous herbivore, considered a cryptic species complex, containing at least 24 morphologically indistinguishable species, and whiteflies populations are called "biotypes" (Barro et al. 2011; Perring 2001). Whiteflies can cause damage to plants in three different ways: (1) suck the sap on leaves via their stylus, (2) produce honeydew facilitating the infection by pathogenic fungi and bacteria, and (3) transmit different plant viruses (Basu 2019). In the case of chili pepper, *Bemisia tabaci* is known to transmit a wide variety of Begomoviruses causing several diseases such as the pepper yellow leaf curl disease or yellow rugosing disease known in Mexico as the "*rizado amarillo*" (De Barro et al. 2008; Maruthi et al. 2007; Morales and Anderson 2001; Padhi et al. 2017). The typical symptoms of virus infection are vein yellowing, foliar yellow mosaics, and leaf curling (Hamilton et al. 1981). Begomoviruses are exclusively transmitted by whiteflies, often in a persistent, circulative, and non-propagative manner (Rosen et al. 2015). By persistence, the virus stays in its vector during its entire life (Nault 1997). The circulative and non-propagative viruses do not replicate inside their vector, but they need to reach the salivary glands to be transmitted through saliva to the plant host when whiteflies ingest the phloem sap (Dietzgen et al. 2016).

1.5.3. Specialist insect

The pepper weevil (*Anthonomus eugenii*, Cano) is a specialist pest of pepper (*Capsicum* spp.) but is also reported to attack other crops in the Solanaceae family, such as eggplants (*Solanum melongena* L.) and the common black nightshade (*Solanum americanum* Mill.) (Capinera 2004; Patrock and Schuster 1992). It is considered a problematic pest of peppers in Mexico, its place of origin, as well as in the Caribbean and Southern United States (Abreu and Cruz 1985; Seal and Martin 2016). Their life-cycle consists of an egg stage, three instars, pupal stage completed entirely inside the fruits, followed by the adult stage when *A. eugenii* exits the fruit by making a hole with their rostrum (Figure 4) (Berdegue et al. 1995; Elmore et al. 1934). Females lay eggs singly in a cavity made with their rostrum (mouthpart), then seals the puncture with a brown fluid secreted through the ovipositor that hardens into a solid egg plug (Elmore and Campbell 1954). Upon hatching, larvae feed on seeds and soft tissue inside the developing fruit, whereas adults feed on flowers and young fruits (Capinera 2014; Costello and Gillespie 1993).

Fig. 4 The life cycle of pepper weevil showing the larval, pupal, and adult stages. All figures © Y. Chabaane.

1.6. Thesis outline

This Ph.D. thesis aimed to study the consequences of domestication of chili pepper on multitrophic interactions, using chili pepper varieties and their wild ancestor Chiltepin as study models (Figure 5).

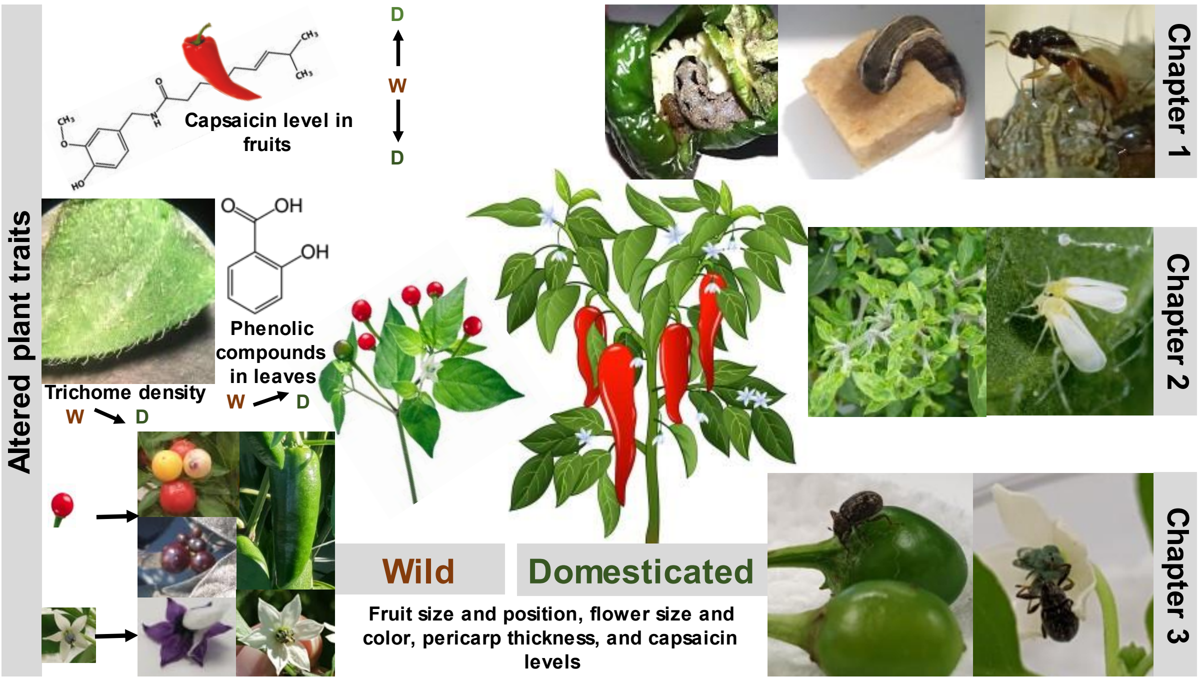


Fig. 5 Summary of the study system.

Chapter one focuses on the effect of altered levels of capsaicin in domesticated chili on the performance of the generalist herbivore *S. latifascia* and its ectoparasitoid *E. platyhypenae.* Although it sounds logical that capsaicin would be toxic for herbivores since it is used as a pesticide. There is surprisingly little evidence for this using fresh fruits, and the effects of capsaicin on the natural enemies of herbivores are not known. To do so, both herbivores and their parasitoids were reared on three chili varieties selected for different pungency levels (Padron= non-pungent, Cayenne= mild, and Habanero= highly pungent) as well as artificial diet spiked with different levels (0, 20, and 200 ppm) of synthetic capsaicin. We found that capsaicin has a strong negative effect on the performance of these two generalist insects, *S. latifascia* and *E. platyhypenae*, especially at high doses. These results increase our understanding of the role of capsaicin as a chemical defense against insects and its potential implications for pest management.

Chapter two explores the pest insects and their associated virus on wild and domesticated chili peppers in their natural environment. For this purpose, we conducted a common garden experiment in Southern Mexico for two successive years, 2019 and 2020. We used three domesticated varieties and three wild accessions of chili pepper planted in an open field. We found that wild chilies were more resistant to chewing insects and virus infection but not to their vector, whitefly. Moreover, chili domestication has altered trichome density and phenolic compounds in leaves. On the one hand, domestication decreased trichome density that could play a role in plant resistance against chewing insects. On the other hand, domesticated plants had more phenolic compounds in leaves than wild chilies, suggesting that these compounds play different roles in the plant, such as protecting chili varieties from potential stress caused by UV radiation. Although it was not the aim of our study, we found that birds selected only fruits from wild accessions. In parallel to the common garden experiment, we sampled wild chilies accessions (Chiltepin) from nine different locations in Southern Mexico. The survey revealed that these plants were strongly resistant to insects and virus damage and that there is a minimal variation in capsaicin levels among these accessions. A better understanding of the natural resistant traits of wild plants altered by domestication could help develop new chili pepper varieties resistant against multiple pests and their associated viruses.

Chapter three is devoted to studying the feeding and oviposition of the specialist pepper weevil (*A. eugenii*) on wild and domesticated chiles as ornamental and for consumption. Studies on this pest focused only on chili varieties selected for cooking. Therefore, the effect of chili domestication on this insect remains unexplored. This study used fruits of one wild accession, Bird pepper Eye, five ornamental varieties (Pops Yellow, Black pearl, Sedona sun, Chilli chilli, and Salsa deep), and two domesticated varieties selected for consumption, Scotch Bonnet and Jalapeño. First, we characterized fruits according to their size, pericarp thickness, capsaicin level, and fruit position. Then, we evaluated the susceptibility of fruits and flowers to attack by *A. eugenii*. Overall, domestication has altered morphological and chemical (capsaicin) traits in fruits with direct consequences for the feeding and oviposition of the pepper weevil. Moreover, we also showed that weevils damage more flowers of the Jalapeño variety than the ornamental Black Pearl pepper or the wild Bird pepper Eye. Our results add to the growing interest in the consequences of crop domestication on herbivores. This knowledge could be integrated into breeding programs aimed at the selection of varieties resistant to this insect.

Finally, in the **general discussion**, the results presented in this thesis are discussed in a broader context.

References

Abreu E, Cruz C (1985) The occurrence of the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) in Puerto Rico Journal of Agriculture of the University of Puerto Rico 69:223-224

Ali JG, Agrawal AA (2012) Specialist versus generalist insect herbivores and plant defense Trends in Plant Science 17:293-302 doi:https://doi.org/10.1016/j.tplants.2012.02.006

Antonious GF, Berke T, Jarret RL (2009) Pungency in *Capsicum chinense*: Variation among countries of origin Journal of Environmental Science and Health Part B 44:179-184 doi:https://doi.org/10.1080/03601230802599118

Atigue MI (2019) L'histoire de la HARISSA avec M. Imed Atigue

Ballina-Gomez H, Latournerie-Moreno L, Ruiz-Sanchez E, Perez-Gutierrez A, Rosado-Lugo G (2013) Morphological characterization of Capsicum annuum L. accessions from southern Mexico and their response to the *Bemisia tabaci*-Begomovirus complex Chilean journal of agricultural research 73:329-338 doi:http://dx.doi.org/10.4067/S0718-58392013000400001

Barro PJD, Liu S-S, Boykin LM, Dinsdale AB (2011) *Bemisia tabaci*: A Statement of Species Status Annual Review of Entomology 56:1-19 doi:10.1146/annurev-ento-112408-085504

Basu A (2019) *Bemisia tabaci* (Gennadius): crop pest and the principal whitefly vector of plant viruses. CRC Press,

Bebbington J, Unerman J (2018) Achieving the United Nations sustainable development goals Accounting, Auditing & Accountability Journal doi:https://doi.org/10.1108/AAAJ-05-2017-2929

Ben Achour M-EA (2020) Mohamed-El Aziz Ben Achour: les Andalous dans l'histoire de Tunisie Leaders,

Benrey B, Callejas A, Rios L, Oyama K, Denno RF (1998) The Effects of Domestication of *Brassica* and *Phaseolus* on the Interaction between Phytophagous Insects and Parasitoids. Biological Control 11:130-140 doi:https://doi.org/10.1006/bcon.1997.0590

Benrey B, Denno RF (1997) The slow-growth–high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987-999. doi:https://doi.org/10.1890/0012-9658(1997)078[0987:TSGHMH]2.0.CO;2

Berdegue M, Harris M, Riley D, Villalon B (1995) Host Plant Resistance on Pepper to the Pepper Weevil, 1991 Arthropod Management Tests 20:365-365

Bosland PW (1996) Capsicums: Innovative uses of an ancient crop Progress in new crops ASHS Press, Arlington, VA:479-487

Boulanger-Fassier S (2013) Le piment d'Espelette AOC: un produit emblématique du territoire basque, une filière dynamique et une notoriété à protéger Sud-Ouest européen Revue géographique des Pyrénées et du Sud-Ouest:97-109 doi:https://doi.org/10.4000/soe.460

Capinera J (2014) Pepper weevil, *Anthonomus eugenii* Cano, and Cuban pepper weevil, *Fausfinus cubae* (Boheman) (Insecta: Coleoptera: Curculionidae) University of Florida EDIS Publication# EENY278(online) http://edis ifas ufl edu/in555 (last accessed 21 Apr 2019)

Capinera JL (2004) Pepper weevil, *Anthonomus eugenii* Cano (Insecta: Coleoptera: Curculionidae) EDIS 2004

Carvalho S, Ragassi C, Bianchetti L, Reifschneider F, Buso G, Faleiro F (2014) Morphological and genetic relationships between wild and domesticated forms of peppers (*Capsicum frutescens* L. and *C. chinense* Jacquin) Genetics and Molecular Research 13:7447-7464

Chang'a A et al. (2016) Scaling-up the use of chili fences for reducing human-elephant conflict across landscapes in Tanzania. Tropical Conservation Science 9:921-930. doi:https://doi.org/10.1177%2F194008291600900220

Chen YH, Gols R, Benrey B (2015a) Crop domestication and its impact on naturally selected trophic interactions. Annual Review of Entomology 60:35-58. doi:https://doi.org/10.1146/annurev-ento-010814-020601

Chen YH, Gols R, Stratton CA, Brevik KA, Benrey B (2015b) Complex tritrophic interactions in response to crop domestication: predictions from the wild. Entomologia Experimentalis et Applicata 157:40-59. doi:https://doi.org/10.1111/eea.12344

Chen YH, Shapiro LR, Benrey B, Cibrián-Jaramillo A (2017) Back to the origin: in situ studies are needed to understand selection during crop diversification. Frontiers in Ecology and Evolution 5:125. doi:https://doi.org/10.3389/fevo.2017.00125

Cichewicz RH, Thorpe PA (1996) The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. Journal of ethnopharmacology 52:61-70. doi:https://doi.org/10.1016/0378-8741(96)01384-0

Costello R, Gillespie D (1993) The pepper weevil, Anthonomus eugenii Cano as a greenhouse pest in Canada the pepper weevil, *Anthonomus eugenii* Cano as a greenhouse pest in Canada 16:31-34

Coudron TA, Kelly TJ, Puttler B (1990) Developmental responses of *Trichoplusia ni* (Lepidoptera: Noctuidae) to parasitism by the ectoparasite Euplectrus plathypenae (Hymenoptera: Eulophidae). Archives of Insect Biochemistry and Physiology 13:83-94. doi: https://doi.org/10.1002/arch.940130108

Cuny MA, Gendry J, Hernández-Cumplido J, Benrey B (2018) Changes in plant growth and seed production in wild lima bean in response to herbivory are attenuated by parasitoids. Oecologia 187:447-457. doi:https://doi.org/10.1007/s00442-018-4119-1

De Barro PJ, Hidayat SH, Frohlich D, Subandiyah S, Ueda S (2008) A virus and its vector, pepper yellow leaf curl virus and *Bemisia tabaci*, two new invaders of Indonesia Biological Invasions 10:411-433 doi:https://doi.org/10.1007/s10530-007-9141-x de Lange ES, Farnier K, Gaudillat B, Turlings TC (2016) Comparing the attraction of two parasitoids to herbivore-induced volatiles of maize and its wild ancestors, the teosintes Chemoecology 26:33-44

Diallo O (2020) Un emblème de la Tunisie, mythique et piquante Harissa TV5monde.

Dietzgen RG, Mann KS, Johnson KN (2016) Plant virus–insect vector interactions: current and potential future research directions Viruses 8:303

Elmore JC, Campbell RE (1954) Control of the pepper weevil.

Elmore JC, Davis AC, Campbell RE (1934) The pepper weevil.

Eshbaugh W (1993) History and Exploitation of a Serendipitous New Crop Discovery. New Crops; Janick, J., Simon, JE, Eds. Wiley: New York, NY, USA,

Fraile A, McLeish MJ, Pagán I, González-Jara P, Piñero D, García-Arenal F (2017) Environmental heterogeneity and the evolution of plant-virus interactions: Viruses in wild pepper populations Virus Research 241:68-76 doi:https://doi.org/10.1016/j.virusres.2017.05.015

Gaillard MD, Glauser G, Robert CA, Turlings TC (2018) Fine-tuning the 'plant domestication reduced defense' hypothesis: specialist vs generalist herbivores. New Phytologist 217:355-366. doi:10.1111/nph.14757

Garvey M, Creighton C, Kaplan I (2020) Pepper domestication enhances parasitoid recruitment to herbivore-damaged plants Arthropod-Plant Interactions 14:695-703 doi:https://doi.org/10.1007/s11829-020-09788-z

Gepts P, Papa R (2001) Evolution during domestication e LS

Gols R, Bukovinszky T, Van Dam NM, Dicke M, Bullock JM, Harvey JA (2008) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. Journal of Chemical Ecology 34:132-143. doi:https://doi.org/10.1007/s10886-008-9429-z

Habib M, Paleari L, Amaral M (1982) Effect of three larval diets on the development of the armyworm, *Spodoptera latifascia* Walker, 1856 (Noctuidae, Lepidoptera). Revista Brasileira de Zoologia 1:177-182. doi:https://doi.org/10.1590/S0101-81751982000300007

Hamilton R, Edwardson J, Francki R, Hsu H, Hull R, Koenig R, Milne R (1981) Guidelines for the identification and characterization of plant viruses Journal of General Virology 54:223-241 doi:https://doi.org/10.1099/0022-1317-54-2-223

Hammer K (1984) Das domestikationssyndrom. Die Kulturpflanze 32:11-34. doi:https://doi.org/10.1007/BF02098682

Hedges S, Gunaryadi D (2010) Reducing human–elephant conflict: do chillies help deter elephants from entering crop fields? Oryx 44:139-146. doi:https://doi.org/10.1017/S0030605309990093

Idris AB, Grafius E (1996) Effects of Wild and Cultivated Host Plants on Oviposition, Survival, and Development of Diamondback Moth (Lepidoptera: Plutellidae) and Its Parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae) Environmental Entomology 25:825-833 doi:https://doi.org/10.1093/ee/25.4.825

Jaccard C, Cuny MA, Bustos-Segura C, Arce C, Giollo L, Glauser G, Benrey B (2021) Squash varieties domesticated for different purposes differ in chemical and physical defense against leaf and root herbivores Frontiers in Agronomy:49 doi:https://doi.org/10.3389/fagro.2021.683936

King AB, Saunders JL (1984) The invertebrate pests of annual food crops in Central America: A guide to their recognition and control. Bib. Orton IICA/CATIE,

Kraft KH, de Jesús Luna-Ruíz J, Gepts P (2013) A new collection of wild populations of Capsicum in Mexico and the southern United States Genetic Resources and Crop Evolution 60:225-232 doi:https://doi.org/10.1007/s10722-012-9827-5

Ladizinsky G (2012) Plant evolution under domestication. Springer Science & Business Media. doi:https://10.1007/978-94-011-4429-2

Liu N, Wei Z, Wei H The effect of planting mode on the growth of pepper in a sunlight greenhouse. In: IOP Conference Series: Earth and Environmental Science, 2021. vol 3. IOP Publishing, p 032001

Maaouia-Houimli SI, Denden M, Dridi-Mouhandes B, Mansour-gueddes SB (2011) Caractéristiques de la croissance et de la production en fruits chez trois variétés de piment (Capsicum annuum L.) sous stress salin Tropicultura 29:75-81

Marina Clemente JA, Palacios Torres RE, Martínez Castro CJ, Javier López L, Aguilera Morales ME (2020) Rentabilidad económica de la producción del chile habanero con mezcla de sustratos y fertilización orgánica en invernadero REPOSITORIO NACIONAL CONACYT

Maruthi MN, Rekha AR, Mirza SH, Alam SN, Colvin J (2007) PCR-based detection and partial genome sequencing indicate high genetic diversity in Bangladeshi begomoviruses and their whitefly vector, Bemisia tabaci Virus Genes 34:373-385 doi:https://doi.org/10.1007/s11262-006-0027-2

McGlashan D, Polston J, Maynard D A survey of viruses affecting Jamaican'Scotch Bonnet'pepper (*Capsicum chinense* Jacq.). In: Proceedings of the Interamerican Society for Tropical Horticulture, 1993. pp 25-30

Meyer RS, DuVal AE, Jensen HR (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. New Phytologist 196:29-48. doi:https://doi.org/10.1111/j.1469-8137.2012.04253.x

Morales FJ, Anderson PK (2001) The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America Archives of Virology 146:415-441 doi:https://doi.org/10.1007/s007050170153

Muniappan R, Bamba J, Cruz J, Reddy G (2004) Biology, rearing and field release on Guam of *Euplectrus maternus*, a parasitoid of the fruit-piercing moth, *Eudocima fullonia*. BioControl 49:537-551. doi:https://doi.org/10.1023/B:BICO.0000036439.74117.2f

Muñoz-Ramírez LS, Peña-Yam LP, Avilés-Viñas SA, Canto-Flick A, Guzmán-Antonio AA, Santana-Buzzy N (2018) Behavior of the hottest chili peppers in the world cultivated in Yucatan, Mexico. HortScience 53:1772-1775. doi:https://doi.org/10.21273/HORTSCI13574-18

Murúa G, Virla EG (2004) Contribution to the biological knowledge of *Euplectrus platyhypenae* (Hymenoptera: Eulophidae), a parasitoid of Spodoptera frugiperda (Lepidoptera: Noctuidae) in Argentina. Folia Entomológica Mexicana 43:171-180

Nakamatsu Y, Tanaka T (2003) Venom of ectoparasitoid, *Euplectrus* sp. near *plathypenae* (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. Journal of Insect Physiology 49:149-159. doi:https://doi.org/10.1016/S0022-1910(02)00261-5

Nakamatsu Y, Tanaka T (2004) Venom of *Euplectrus separatae* causes hyperlipidemia by lysis of host fat body cells. Journal of Insect Physiology 50:267-275. doi:10.1016/j.jinsphys.2003.12.005

Nasruddin A et al. (2020) Effects of Mulch Type, Plant Cultivar, and Insecticide Use on Sweet Potato Whitefly Population in Chili Pepper Scientifica 2020:6428426 doi:https://doi.org/10.1155/2020/6428426

Nault LR (1997) Arthropod Transmission of Plant Viruses: a New Synthesis Annals of the Entomological Society of America 90:521-541 doi:10.1093/aesa/90.5.521

Omolo MA et al. (2014) Antimicrobial properties of chili peppers. Journal of Infectious Diseases and Therapy 2:145. doi:10.4172/2332-0877.1000145

Opitz SE, Müller C (2009) Plant chemistry and insect sequestration. Chemoecology 19:117-154. doi:https://doi.org/10.1007/s00049-009-0018-6

Othmani W (2020) Intangible heritage as a social construction of authenticity: The example of Tunisian cuisine

Padhi GK, Maity L, Chattopadhyay A, Samanta A (2017) Population dynamics of whitefly (Bemisia tabaci Genn.) in chilli and screening of genotypes against chilli leaf curl virus J Entomol Zool Stud 5:104-107

Pandhair V, Sharma S (2008) Accumulation of capsaicin in seed, pericarp and placenta of Capsicum annuum L fruit Journal of Plant Biochemistry and Biotechnology 17:23-27

Patrock R, Schuster D (1992) Feeding, oviposition and development of the pepper weevil, (Anthonomus eugenii Cano), on selected species of Solanaceae International Journal of Pest Management 38:65-69

Pereira-Dias L, Vilanova S, Fita A, Prohens J, Rodríguez-Burruezo A (2019) Genetic diversity, population structure, and relationships in a collection of pepper (Capsicum spp.) landraces from the Spanish centre of diversity revealed by genotyping-by-sequencing (GBS) Horticulture research 6:1-13 doi:https://doi.org/10.1038/s41438-019-0132-8

Perramond E (2005) The politics of ecology: local knowledge and wild chili collection in Sonora, Mexico Journal of Latin American Geography:59-75

Perring TM (2001) The *Bemisia tabaci* species complex Crop Protection 20:725-737 doi:https://doi.org/10.1016/S0261-2194(01)00109-0

Perry L, Flannery KV (2007) Precolumbian use of chili peppers in the Valley of Oaxaca, Mexico Proceedings of the National Academy of Sciences 104:11905-11909 doi:https://doi.org/10.1073/pnas.0704936104

Pickersgill B (2007) Domestication of Plants in the Americas: Insights from Mendelian and Molecular Genetics Annals of Botany 100:925-940 doi:https://doi.org/10.1093/aob/mcm193

Pickersgill B (2016) Chile peppers (*Capsicum* spp.). In: Ethnobotany of Mexico. Springer, pp 417-437. doi:https://doi.org/10.1007/978-1-4614-6669-7_17

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual review of Ecology and Systematics 11:41-65 doi:https://doi.org/10.1146/annurev.es.11.110180.000353

Qureshi J, Kostyk BC (2020) Insecticidal Control of Pepper Weevil on Jalapeño Pepper, Spring 2019 Arthropod Management Tests 45:tsaa027

Rosen R et al. (2015) Persistent, circulative transmission of begomoviruses by whitefly vectors Current Opinion in Virology 15:1-8 doi:https://doi.org/10.1016/j.coviro.2015.06.008

Rowell-Rahier M, Pasteels JM (1992) Third trophic level influences of plant allelochemics. In: Herbivores: their interactions with secondary plant metabolites, vol 2. vol 5. Elsevier, pp 243-277

Safari M, Roossinck MJ (2018) Coevolution of a persistent plant virus and its pepper hosts Molecular Plant-Microbe Interactions 31:766-776 doi:https://doi.org/10.1094/MPMI-12-17-0312-R

Sassi M, Sassi, Acocella (2018) Understanding Food Insecurity. Springer,

Scoville WL (1912) Note on capsicums. Journal of the American Pharmaceutical Association 1:453-454. doi:https://doi.org/10.1002/jps.3080010520

Seal DR, Martin CG (2016) Pepper weevil (Coleoptera: Curculionidae) preferences for specific pepper cultivars, plant parts, fruit colors, fruit sizes, and timing. Insects 7:9 doi:https://doi.org/10.3390/insects7010009

Shlichta JG, Cuny MA, Hernandez-Cumplido J, Traine J, Benrey B (2018) Contrasting consequences of plant domestication for the chemical defenses of leaves and seeds in lima bean plants. Basic and Applied Ecology 31:10-20. doi:https://doi.org/10.1016/j.baae.2018.05.012

Stommel J, Bosland P (2006) Pepper, ornamental, *Capsicum annuum* Flower breeding and genetics: Issues, challenges and opportunities for the 21st century Springer, Dordrecht, The Netherlands:561-599 doi:https://doi.org/10.1007/978-1-4020-4428-1_21

Swezey O (1924) The Mexican armyworm parasite (*Euplectrus platyhypenae*). Hawaii planters' record 28:318-320

Tong N, Bosland P (2004) *Capsicum tovarii*, a new member of the *Capsicum baccatum* complex Euphytica 109:71-77 doi:10.1023/A:1003421217077

Traine J, Cuny MA, Bustos-Segura C, Benrey B (2020) The interaction between host and host plant influences the oviposition and performance of a generalist ectoparasitoid. Entomologia Experimentalis et Applicata 169:133-144. doi:https://doi.org/10.1111/eea.12949

Turcotte MM, Turley NE, Johnson MT (2014) The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. New Phytologist 204:671-681. doi:https://dx.doi.org/10.1111/nph.12935

Turlings TC, Benrey B (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. Ecoscience 5:321-333. doi:https://doi.org/10.1080/11956860.1998.11682472

Turlings TC, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251-1253. doi:https://doi.org/10.1126/science.250.4985.1251

Vet LE, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annual review of entomology 37:141-172. doi:https://doi.org/10.1146/annurev.en.37.010192.001041

Walsh B, Hoot S (2001a) Phylogenetic Relationships of Capsicum (Solanaceae) Using DNA Sequences from Two Noncoding Regions: The Chloroplast atpB - rbcL Spacer Region and Nuclear waxy Introns Int J Plant Sci 162:1409-1418 doi:10.1086/323273

Walsh BM, Hoot SB (2001b) Phylogenetic relationships of Capsicum (Solanaceae) using DNA sequences from two noncoding regions: the chloroplast atpB-rbcL spacer region and nuclear waxy introns International Journal of Plant Sciences 162:1409-1418

Whitehead SR, Turcotte MM, Poveda K (2017) Domestication impacts on plant–herbivore interactions: a meta-analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 372:20160034. doi:https://doi.org/10.1098/rstb.2016.0034

Wu P, Haseeb M, Zhang R, Kanga LHB, Legaspi JC (2019) In vitro consumption patterns of pepper weevil, Anthonomus eugenii (Coleoptera: Curculionidae) on two commercial pepper cultivars in Florida Applied Entomology and Zoology 54:473-479 doi:https://doi.org/10.1007/s13355-019-00645-x

Zagatti P, Lalanne-Cassou B, le Duchat d'Aubigny J (1995) Catalogue of the Lepidoptera of the French Antilles. INRA

2. Altered capsaicin levels in domesticated chili pepper varieties affect the interaction between a generalist herbivore and its ectoparasitoid

Yosra Chabaane[1], Carla Marques Arce[2], Gaëtan Glauser[3] and Betty Benrey[1*]

[1]Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

[2]Fundamental and applied research in chemical ecology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

[3]Neuchâtel platform of analytical chemistry, Institute of Chemistry, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

[*]Author for correspondence (e-mail: betty.benrey@unine.ch)

Published in the Journal of Pest Science

25 June 2021

https://link.springer.com/article/10.1007/s10340-021-01399-8

https://doi.org/10.1007/s10340-021-01399-8

Abstract

Plant domestication has commonly reduced levels of secondary metabolites known to confer resistance against insects. Chili pepper is a special case because the fruits of different varieties have been selected for lower and higher levels of capsaicin, the main compound associated with defence. This may have important consequences for insect herbivores and their natural enemies. Despite the widespread consumption of chili peppers worldwide, the effects of capsaicin on insects are poorly understood. Here, we investigated the effect of capsaicin on a generalist herbivore, *Spodoptera latifascia* (Lepidoptera: Noctuidae) and its ectoparasitoid, *Euplectrus platyhypenae* (Hymenoptera: Eulophidae). Using chili varieties with three pungency levels: non-pungent (Padron), mild (Cayenne) and highly pungent (Habanero), as well as artificial diets spiked with three different levels of synthetic capsaicin, we determined whether higher capsaicin levels negatively affect the performance of these insects. Overall, capsaicin had a negative effect on both herbivore and parasitoid performance, particularly at high concentrations. Caterpillars reared on highly pungent fruits and high-capsaicin diet had longer development time, reduced pupation success, lower adult emergence, but also lower parasitism rates than caterpillars reared on mild or non-capsaicin treatments. In addition, we found that the caterpillars were capable of sequestering capsaicinoids in their haemolymph when fed on the high pungent variety with consequences for parasitoids' performance and oviposition decisions. These results increase our understanding of the role of capsaicin as a chemical defence against insects and its potential implications for pest management.

Keywords Chili pepper · Domestication · Capsaicinoids · Plant-mediated · Sequestration · Tritrophic interactions.

Key message

- Domestication of chili peppers has altered the content of capsaicin in fruits, which is responsible for the chili's pungency.

- Capsaicin has been associated with defense against insects, but the evidence is limited.

- We tested the effects of capsaicin on a generalist herbivore and its parasitoid.

- Capsaicin had negative effects on herbivore performance, cascading up to the parasitoid.

- The results support the role of capsaicin as a chemical defense against insects, with possible implications for pest management.

2.1. Introduction

Plant domestication has resulted in a suite of morphological, nutritional and chemical traits that distinguish crops from their wild counterparts (Gepts 2004; Smartt and Simmonds 1995). One of the main changes in crops is a reduction in secondary metabolites (Meyer et al. 2012). The reason for this change is to render plants more suitable for human consumption (Ladizinsky 2012). In this context, chili pepper (*Capsicum* spp., family Solanaceae) offers a unique model to examine the relationship between domestication and altered levels of chemical defences.

The genus *Capsicum* is resolved as a monophyletic group with five domesticated taxa and around 20–30 wild species (Carrizo García et al. 2016). In contrast to most crop plants for which domestication has resulted in a decrease of secondary metabolites, chili peppers have been selected for both increased and decreased levels of their main secondary metabolites so-called capsaicinoids (Aza-González et al. 2011; Kim 2014) as compared to the wild ancestor, Chiltepin (González-Zamora et al. 2015; Tewksbury et al. 2008). These secondary compounds are biosynthesized and accumulated in the placenta tissue and responsible for the pungency or spiciness in chili fruits (Pickersgill 2016). The varietal selection was performed along a gradient from low to high pungency (from 0 to 1,500,000 Scoville units) (Scoville 1912). The main capsaicinoids are capsaicin and dihydrocapsaicin which represent 90% of the whole capsaicinoids in fruits (Govindarajan and Salzer 1985). Capsaicinoids are synthetized via two different pathways, the phenylpropanoid and the branched-chain fatty acid pathways (Aza-González et al. 2011), and two genes were identified to be responsible for chili pepper pungency, Pun1 and pAMT located, respectively, on chromosome 2 and chromosome 3 (Lang et al. 2006; Stewart Jr et al. 2005). The unfunctional alleles of these genes cause the loss of pungency found in sweet pepper varieties (Tsurumaki and Sasanuma 2019).

Capsaicinoids are known to have deterrent and medicinal properties for mammals. For example, chili has been used as a crop guarding system in different African and Asian countries to reduce human–elephant conflicts (Chang'a et al. 2016; Hedges and Gunaryadi 2010). Interestingly, birds are not sensitive to capsaicin (Mason and Maruniak 1983; Szolcsányi et al. 1986). A comparison between chicken capsaicin vanilloid receptors (cTRPV19) and its rat counterparts (rTRPV1) showed a high structural divergence (only 68% amino acid identity) (Jordt and Julius 2002). The difference between both vanilloid receptors might be the result of selective pressures that facilitate the differentiation of the ecological niche of each species. Indeed, it has been suggested that birds evolved as vectors for fruit dispersion whereas mammals were repelled to avoid the destruction of seeds (Tewksbury and Nabhan 2001; Tewksbury et al. 2008). Chili has been also known for its medicinal uses long before the Spanish colonization of the Americas and since the Mayan civilization (Cichewicz and Thorpe 1996; Pickersgill 2016; Witting et al. 2000).

The effect of capsaicin on pathogenic bacteria and fungi has also been widely studied. Capsaicin inhibits and retards the growth of several human (e.g. *Helicobacter pylori*, *Escherichia coli*, *Streptococcus pyogenes*), soil (e.g. *Bacillus subtilis* and *Pseudomonas solanacearum*) and plant pathogenic (e.g. *Xanthomonas campestris*, *Pseudomonas syringae*) bacteria (Argaez et al. 2009; Jones et al. 1997; Marini et al. 2015; Molina-Torres 1999). For example, non-pungent wild chili fruits were twice more infested by *Fusarium* spp. As compared to wild pungent fruits, suggesting that capsaicinoids protect fruits from pathogenic fungi (Haak et al. 2012; Tewksbury et al. 2008). Likely, the reason why hot chilies have been used for food preservation in many regions, long before the use of refrigerators (Omolo 2014).

For insects, there is a common assumption that capsaicin is toxic. Several studies have shown that capsaicin deters oviposition (Cowles et al. 1989), slows down larval development (Ahn et al. 2011a; Weissenberg et al. 1986) and inhibits feeding (Hori et al. 2011). Moreover, synthetic capsaicin has even been used as a pesticide against some insect pests (wilsonKoleva-Gudeva et al. 2013; Wilson 1996). Although the above studies demonstrate the negative effects of capsaicin on insect herbivores, they were all conducted using either artificial diet in which pure capsaicin or dried chili powder was added. As yet, only a handful study has examined the effect of capsaicin on insect herbivores using fresh fruits (Tęgowska et al. 2005). Plant secondary metabolites can also affect the natural enemies of herbivores in different ways (Chen et al. 2015; Turlings and Benrey 1998). For example, parasitoid wasps can benefit from the volatiles emitted from herbivore-damaged plants and use them as cues to find their host (Turlings et al. 1990; Vet and Dicke 1992). In addition, secondary metabolites can slow down herbivore development and thus increase their time of exposure to natural enemies (Benrey and Denno 1997; Price et al. 1980). Alternatively, some herbivores can sequester and store plant toxins in their bodies making them unpalatable or toxic for their natural enemies (Opitz and Müller 2009; Rowell-Rahier and Pasteels 1992). For example, El-Heneidy et al. (1988) found that the survival of an Ichneumonid parasitoid (*Hyposoter annulipes*) (Hymenoptera: Ichneumonidae) was reduced when its larval host, the fall armyworm (*Spodoptera frugiperda*) (Lepidoptera: Noctuidae), was fed on artificial diet mixed with nicotine. In another study, it was shown that by sequestering alkaloids from its host plant, larvae of the sawfly (*Rhadinoceraea nodicornis*) (Hymenoptera, Tenthredinidae) were protected against generalist predators (Schaffner et al. 1994). To date, however, the effects of capsaicin on the natural enemies of herbivores are not known. Knowing whether capsaicin has a negative effect on insect herbivores and these effects cascade up to their natural enemies is a valuable information for pest management practices of chili pepper.

The aim of this study was to examine how altered capsaicin levels in chili peppers as a result of varietal selection affect the tritrophic interaction with a generalist herbivore *Spodoptera latifascia* (Lepidoptera: Noctuidae) and one of its larval ectoparasitoid, *Euplectrus platyhypenae* (Hymenoptera: Eulophidae). We used a combination of chemical analyses and behavioural assays to address the following questions: (1) is capsaicin toxic for these insects? and (2) what are the direct and indirect (via the host caterpillar) effects of capsaicin on herbivore and parasitoid performance? To answer these, first we determined capsaicin levels in fruits of three varieties selected for different pungency levels. Secondly, we reared herbivores on fruits of these varieties, as well as parasitoids on caterpillars fed with these fruits and determined their performance, and finally, we reared parasitized and non-parasitized caterpillars on artificial diet with different levels of synthetic capsaicin.

Our results provide insight into how varietal selection of this important crop has influenced its interactions with herbivores and their parasitoids. Furthermore, to our knowledge, this is the first time that the effects of capsaicin on the third trophic level are examined.

2.2. Material and methods

2.2.1. Chili fruits

Chili fruits were purchased from a local market in Neuchâtel, Switzerland. We selected three varieties based on their known pungency level: non-pungent variety Padron (*C. annuum*), mild variety Cayenne (*C. annuum*) and highly pungent variety, Habanero (*C. chinense*). These varieties are originally from Latin America (Muñoz-Ramírez et al. 2018), except for Padron, which was selected in Galicia, Spain (Katz 2009). These varieties were used in all the experiments with fruits.

2.2.2. Insects

Spodoptera latifascia, commonly known as the Velvet armyworm, occurs naturally throughout Mexico and Central America (Saunders et al. 1988; Zagatti et al. 1995). It is a polyphagous insect whose host range includes several crops such as potato, cotton, soybeans, maize, and beans (Cuny et al. 2018; Habib et al. 1982). Larvae have been frequently found feeding on leaves of chili plants in Mexico (Traine et al. 2020).

Euplectrus platyhypenae is a gregarious koinobiont ectoparasitoid, originated from Mexico, and parasitizes third and fourth instars of Noctuid and Geometrid caterpillars (Muniappan et al. 2004; Murúa and Virla 2004; Swezey 1924). Prior to oviposition, female wasps inject a venom on the dorsum of the caterpillar to inhibit molting without killing their host. One female lays up to 20 eggs that develop on the dorsal segments of the host's body feeding on its haemolymph (Coudron et al. 1990; Nakamatsu and Tanaka 2003). Before pupation, the parasitoid larvae use their saliva to kill the host and move underneath the cadaver to pupate (Nakamatsu and Tanaka 2004). Approximately one-week later adults emerge (Nakamatsu and Tanaka 2003).

Colonies of *S. latifascia* and *E. platyhypenae* were established with insects originally collected from beans, squash, and chili pepper in the experimental campus of the Universidad del Mar, in Puerto Escondido, (Oaxaca, Mexico; 15°55′33.3″N, 97°09′03.0″W). Then, they were reared at the University of Neuchâtel under quarantine conditions, level 3 (26 °C, 60% relative humidity and L12:D12). Caterpillars were fed on artificial diet (soy-wheat germ diet, Frontier scientific services, USA) in plastic boxes (13 × 15 × 5 cm) with fabric mesh for aeration. Parasitoids were reared on two different species, *S. latifascia* and *S. frugiperda* third instar caterpillars, which were fed with artificial diet and maize leaves were offered to maximize caterpillar survival until parasitoid pupation. Parasitoid adults were kept in 30 × 30 × 30 cm mesh cages (Bioquip Products) with water and honey as food source.

2.2.3. Quantification of capsaicinoids in chili fruits and herbivore haemolymph

To verify the pungency level of the fruits of the selected varieties, we quantified the capsaicinoids content in chili fruits. Whole fruits were oven dried for 48 h at 60 °C following the method developed by Collins et al. (1995). Once dried, each fruit was ground separately with a mortar to obtain a fine powder and 10 mg was extracted with 1 ml of methanol. The mixture was then centrifuged for 5 min at 14 000 rpm, 700 ul of supernatant was collected and further diluted 100′000-fold prior to HPLC analysis. For each variety, we had 10 replicates (1 replicate = 1 fruit).

To investigate whether capsaicinoids can be sequestered to other tissues apart from the gut, we measured the capsaicin content in the haemolymph of *S. latifascia* caterpillars fed for 7 days on the three chili fruit varieties (non-pungent variety Padron, mild variety Cayenne and highly pungent variety Habanero). The haemolymph was collected by puncturing the cuticle at the dorsal part of the thorax. From each larva, we collected 2 µl of haemolymph exuded immediately after the incision in an Eppendorf tube containing 10 µl of an anticoagulant solution composed of 98 mM NaOH, 186 mM NaCl, 17 mM Na2EDTA and 41 mM citric acid (pH 4.5) (Haine et al. 2007). We had five replicates per treatment and each replicate was a pool of two caterpillars. Then, 400 µl of methanol (100%) was added to each sample that were centrifuged for 5 min at 14 000 rpm and the supernatants were filtered using a hydrophilic PTFE filter (size = 13 mm; Thermo Fisher Scientific) and a single-use-syringe (1 ml, Soft Ject). The samples were diluted 1000-fold with methanol (100%) prior to analysis and they were kept at − 80 °C until further analysis.

The capsaicinoid content was analysed using an Acquity ultra-high-pressure liquid chromatography (UHPLC) system coupled to a Synapt G2 QTOF mass spectrometer (Waters, Milford, MA, USA) controlled by Masslynx 4.1. The separation was performed on a Waters Acquity BEH C18 column (50 × 2.1 mm i.d., 1.7 µm particle size) thermostated at 25 °C. Mobile phases consisted of water containing 0.05% formic acid (solvent A) and acetonitrile containing 0.05% formic acid (solvent B). Standards of capsaicin (> 95% from *Capsicum* spp,) and dihydrocapsaicin (> 85% from *Capsicum* spp.) from Sigma-Aldrich (St. Louis, Missouri) were used to identify and quantify capsaicinoids in fruits and in the haemolymph of *S. latifascia* caterpillars. Standard curves were prepared using concentrations of 0.04, 0.2, 1 and 5 ug/ml.

2.2.4. Bioassays

To investigate the impact of capsaicin on the performance of the herbivore *S. latifascia* and the parasitoid *E. platyhypenae*, we fed caterpillars separately on chili fruits and capsaicin-spiked artificial diet.

- **Effect of pungency level on the performance of the herbivore *Spodoptera latifascia***

Caterpillars were fed on mature chili fruits from three different varieties: non-pungent variety Padron, mild variety Cayenne and highly pungent variety Habanero. Three-day old larvae were individually placed, with a piece of fruit containing the placenta part, in a small cylindrical plastic container (0.23 L) covered with mesh for aeration. New fresh fruits were provided for larvae every other day. The number of replicates (plastic containers) used for each variety is as follows: non-pungent = 20, mild = 18, and highly pungent = 17. The parameters recorded were: caterpillars weight until they pupate, pupation (number of pupae/number of remaining larvae) *100), pupal weight measured 24 h after pupation, and adult emergence (number of adults/ number of remaining larvae)*100). The measurements were taken every three days for caterpillar weight until pupation and then every day until adult emergence. Larval and pupal weights were measured using an electronic balance (BP 161P, Sartorius, Goettingen, Germany).

To determine the effect of capsaicin alone, without other possible effects of the chili fruits, we conducted a parallel experiment with artificial diet (Soy-wheat germ diet, Frontier scientific services) spiked with capsaicin. Larvae were reared on diet with three different levels of synthetic capsaicin to mimic the gradient of pungency used for the chili fruit experiment: control (without capsaicin), low-capsaicin (20 ppm = 0,02 mg g^{-1}) and high-capsaicin diet (200 ppm = 0,2 mg g^{-1}). The number of replicates used for each capsaicin treatment is as follows: no-capsaicin = 17, 20 ppm = 19, and 200 ppm = 18. The capsaicin-spiked diet for the feeding experiment was prepared by adding two different concentrations of capsaicin (≥ 95% from Capsicum spp. from Sigma-Aldrich, Switzerland) dissolved in ethanol and mixed at 20 ppm and 200 ppm with the diet before solidification. For the control treatment, only ethanol was added. Due to the very irritating nature of pure capsaicin, we could not mimic the exact levels as found in Habanero fruits. However, we used concentrations of capsaicin that have been proven effective in other studies with Noctuidae species (Ahn et al. 2011a). We carried out this experiment using the same protocol as for the experiment with fruits (Sect. 1a).

- **Effect of pungency level on the performance of the parasitoid *Euplectrus platyhypenae***

To investigate whether the pungency level in chili fruit affects the third trophic level, the performance of *E. platyhypenae* was assessed when reared on *S. latifascia* larvae fed on the three chili varieties (Padron = non-pungent, Cayenne = mild and Habanero = highly pungent). For the control treatment (N = 10), larvae were fed on a maize leaf with a piece of artificial diet to assure optimal oviposition by the wasp *E. platyhypenae* (Traine et al. 2020).

Caterpillars were reared individually for 6 days on the three varieties. On day seven, one fourth-instar caterpillar was placed on a piece of fruit from its rearing variety and placed in a 9 × 2 cm Petri dish. Subsequently, one couple of *E. platyhypenae* were introduced in the Petri dish. The food source for the caterpillars (host) was present during the whole period of exposure to the parasitoids. Thirteen replicates (Petri dishes) were used for each variety. The average time for parasitism was between three and four days. As soon as the first clutch of eggs was observed on the larvae, the adults of *E. platyhypenae* were removed. Afterward, we recorded parasitism (number of parasitized larvae/number of remaining larvae)*100) and the clutch size defined as the number of eggs laid in a single reproductive bout (Godfray 1994). For each parasitized larva, the number of eggs laid by the wasp was counted using a hand lens (Triplet, 30X-21 mm).

In a parallel experiment, we followed the same procedure but removed the fruits and replaced them with no-capsaicin artificial diet when the caterpillars (host) were exposed to the parasitoids. This allowed us to test whether female wasps were capable of perceiving the capsaicin present in the host haemolymph. We recorded the same parameters as in the previous experiment. Five replicates (Petri dishes) were used for each variety.

The goal of this experiment was to determine the effect of capsaicin alone on the parasitoid response independent of other potential fruit factors. We used artificial diet spiked with capsaicin at three different concentrations 0, 20 and 200 ppm. The same protocol as for the experiment with fruits was used. In a first experiment, larvae were fed on their original capsaicin-spiked diet (capsaicin diet) before and after the exposure to the wasps. In a second experiment, the capsaicin-spiked diet was replaced by a common diet without capsaicin (no-capsaicin diet) only when caterpillars were exposed to the wasp. The purpose of these two different designs was to examine whether female wasps can perceive the capsaicin present in the host haemolymph independently when fruits were not present. In both experiments, five replicates (Petri dishes) were used for each capsaicin treatment.

2.2.5. Statistical analysis

All statistical analyses were performed in R statistical software (version 3.5.3; R Development Core Team 2020) by using ANOVA, followed by residual analysis to verify suitability of distributions of the tested models. To test the effect of capsaicin on caterpillars' weight, when feeding on either chili fruits or on artificial diet spiked with synthetic capsaicin, generalized linear mixed models (GLMMs) with a Gaussian distribution were used. GLMMs included 'treatment', 'time' and the interactions between 'treatment' and 'time', replicate and time as random factors. Least squares means (LSMeans) were used to compare significantly differences among treatments. Generalized linear models (GLMs) with a Gaussian distribution were used to verify the pupal weight, parasitoid clutch size, capsaicinoid contents in fruits and haemolymph. Least squares means (LSMeans) were used to compare significantly differences among treatments. Parasitoid emergence, herbivore pupation rate and adult emergence were analysed using generalized linear models (GLM) under binomial distribution. The effects of treatments on caterpillar's pupation time were analysed using the package "Survival" from R under Weibull distribution. The overdispersion of the data was verified and if necessary, the correction by using quasibinomial was applied. The sample size and number of replicates for all experiments are indicated directly in figure captions.

2.3. Results

2.3.1. Quantification of capsaicinoid in fruits

Capsaicinoid analysis of the chili fruits showed considerable quantitative variation among the three chili varieties both in capsaicin and dihydrocapsaicin levels (Fig. 1, capsaicin F[2,33] = 79.138, d.f = 2, $P < 0.001$ and dihydrocapsaicin; F[2,33] = 73.585, d.f = 2, $P < 0.001$). The Habanero variety had 11 and 22 times more capsaicin and dihydrocapsaicin (capsaicin: 17.89 mg g^{-1} of dry weight (DW); dihydrocapsaicin: 11.41 mg g^{-1} DW), respectively, than the mild Cayenne variety (capsaicin: 1.53 mg g^{-1} DW; dihydrocapsaicin: 0.51 mg g^{-1} DW), whereas the Padron variety had no capsaicinoids at all (Fig. 1). In the case of Habanero and Cayenne, capsaicin content was around 1.5 and 3 times higher than dihydrocapsaicin, respectively (Fig.1).

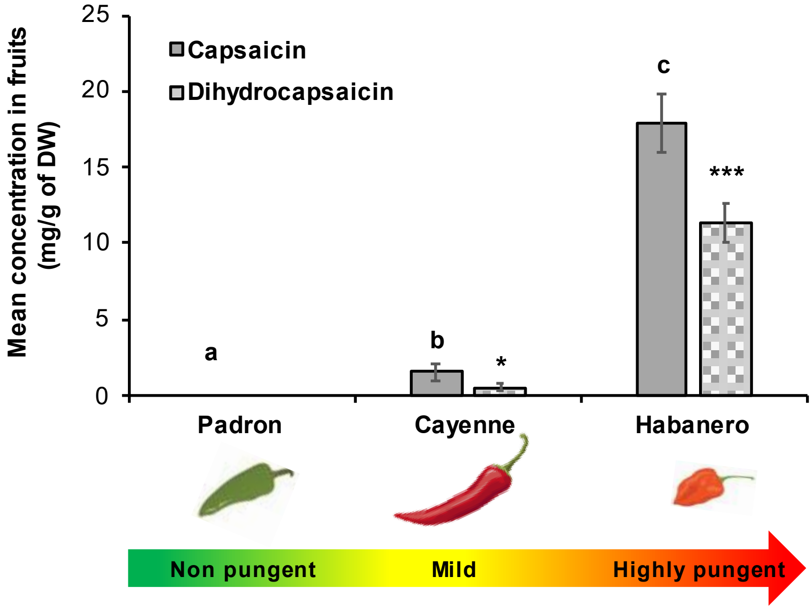


Fig. 1 Mean (± SEM) capsaicinoids content (mg/g of DW) in the three chili varieties, Padron, Cayenne and Habanero. Difference among treatments is indicated by different letters for capsaicin and stars for the dihydrocapsaicin concentrations (F-test, Tukey post hoc test with Bonferroni correction: $P < 0.001$, N = 10).

2.3.2. Effect of pungency level on the performance of the herbivore *Spodoptera latifascia*

The performance of caterpillars of *S. latifascia* was negatively affected by the pungency levels in the fruits. Caterpillars grew larger (Fig. 2a, $\chi2 = 47.492$, d.f = 2, $P < 0.001$) on the non-pungent variety than on mild and highly pungent fruits. Moreover, caterpillars took only 27 days to pupate in the non-pungent variety compared to 33 and 45 days in the mild and highly pungent varieties, respectively (Fig. 2a, $\chi2 = 45.95$, d.f = 2, $P < 0.001$).

Feeding on mild and highly pungent varieties significantly reduced the pupation rate compared to feeding on non-pungent chili fruits (Fig. 2b, $\chi2 = 62.160$, d.f = 2, $P < 0.001$). However, no difference among the treatments was found for the weight of pupae (Supplementary Table 1, F[2, 21] = 0.1167, d.f = 2, $P = 0.89$). The percentage of adult emergence for *S. latifascia* was significantly higher on the non-pungent Padron variety than on the mild variety (Fig. 2c, $\chi2 = 38.213$, d.f. = 2, $P < 0.001$). On the highly pungent variety, the pupae did not reach the adult stage (Fig. 2c).

Capsaicin-spiked diet did not have a significant effect on the larval weight of *S. latifascia* caterpillars (Fig. 2d, $\chi2 = 4.6453$, d.f = 2, $P = 0.96$). However, pupation rate and adult emergence were negatively affected at the higher concentration of 200 ppm (Fig. 2e, $\chi2 = 60.478$, d.f = 2, $P = 0.009$ and Fig. 2f, $\chi2 = 58.261$, d.f = 1, $P = 0.001$). Caterpillars that fed on a diet containing 200 ppm of capsaicin pupated on average 35% less than caterpillars on the capsaicin-free and 20-ppm diets (Fig. 2e). Moreover, 30% and 41% fewer adults emerged from the 200-ppm diet treatment than from the no-capsaicin and 20-ppm treatments (Fig. 2f).

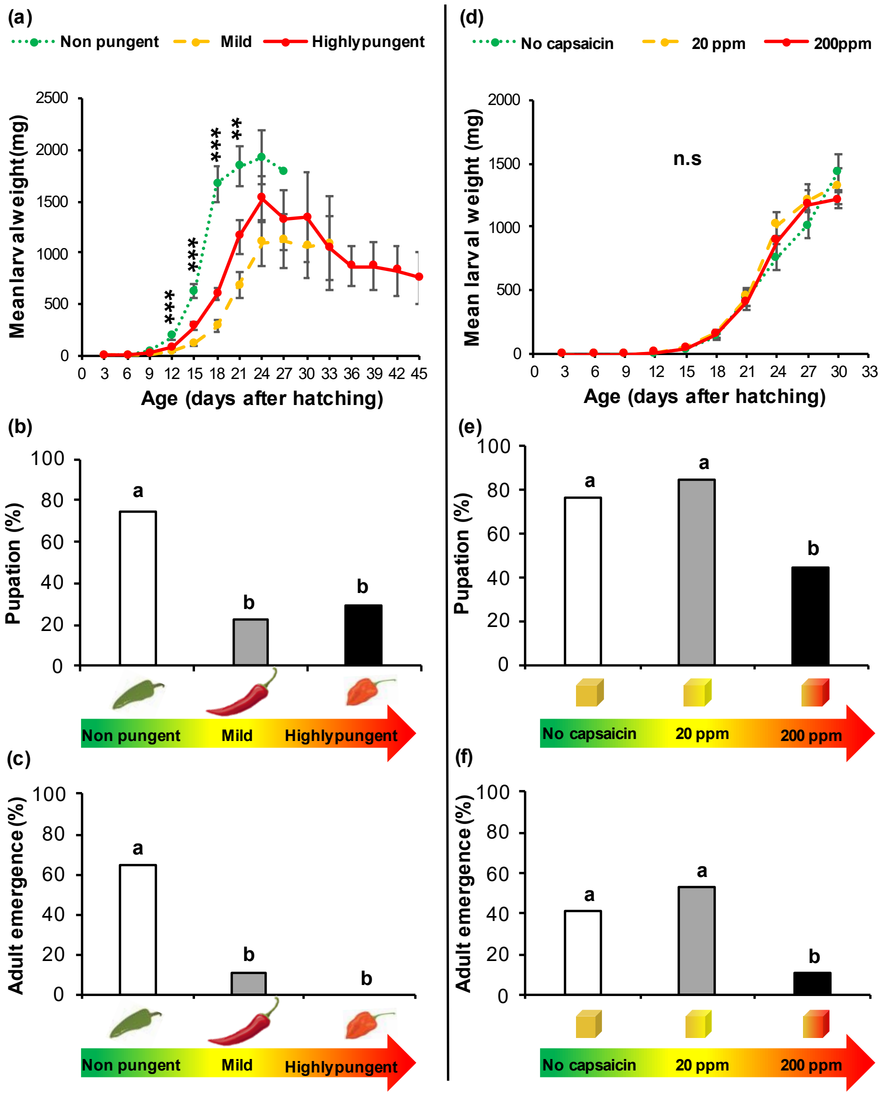


Fig. 2 Effect of pungency level (in fruits) and capsaicin content (in diet) on the performance of *Spodoptera latifascia*: **a** mean larval weight (mg), **b** pupation (%) and **c** adult emergence (%) of *S. latifascia* feeding on chili fruits with three different pungency levels non-pungent, mild, and highly pungent, **d** mean larval weight (mg),**e** pupation (%) and **f** adult emergence (%) of caterpillars feeding on artificial diet mixed with three levels of synthetic capsaicin (no capsaicin, 20 and 200 ppm). Means in a for the same age capped with asterisks are significantly different (F-test, Tukey post hoc test with Bonferroni correction: ** $P < 0.01$, ***$P < 0.001$). Different letters indicate a significant difference between treatments for pupation and adult emergence (Chi-test, Tukey post hoc test with Bonferroni correction: $P < 0.01$). Sample sizes: Fig. 2a, b and c, non-pungent = 20, mild = 18 and highly pungent = 17; Fig. 2d, e and f, no-capsaicin = 17, 20 ppm = 19 and 200 ppm = 18

2.3.3. Effect of pungency level on the performance of *Euplectrus platyhypenae*

The pungency level in fruits had a negative effect on parasitism rate (Fig. 3a, b). Parasitism rate was 30% lower on caterpillars reared on the highly pungent Habanero variety in the presence of fruits (Fig. 3a, $\chi2 = 57.196$, d.f = 3, $P = 0.06$) and 80% lower on caterpillars reared on this same variety but exposed to the wasps without the fruits (Fig. 3b, $\chi2 = 11.506$, d.f = 3, $P = 0.003$) than on caterpillars reared on the other two varieties. There was no significant difference on parasitism rate between mild and non-pungent treatments in the presence and absence of chili fruit (Fig. 3a, b). In the absence of the chili fruit when exposed to the wasps (Fig. 3b), parasitism rates for both mild and non-pungent treatments were as high as for the control, whereas when chili fruits were present, the parasitism rate for the same treatments was around 30% lower than the control (Fig. 3a).

Pungency level had no significant effect on the clutch size laid by *E. platyhypenae* on *S. latifascia* (Supplementary Fig. 2a, F[3, 27] = 0.4695, d.f = 3, *P* = 0.70, Supplementary Fig. 2b, F[3, 16] = 0.5711, d.f = 3, *P* = 0.64).

We found similar results for parasitoids when exposed to caterpillars reared on capsaicin-spiked diets. Parasitism rate was approximately 60% lower on larvae from the high capsaicin diet (200 ppm) than on larvae from the other two treatments (no capsaicin) and 20 ppm (Fig. 3c, $\chi2$ = 16.510, d.f = 3, P = 0.011). However, in the second experiment when larvae from the three spiked-diet treatments were switched to a non-spiked artificial diet and then exposed to parasitism, this protection was lost (Fig. 3d. $\chi2$ = 6.5017, d.f = 3, *P* = 0.59).

No significant difference among treatments was found for clutch size (Supplementary Fig. 2c, F[3, 15] = 3.037, d.f = 3, *P* = 0.061; Supplementary Fig. 2d, F[3, 20] = 1.1686, d.f = 3, *P* = 0.34).

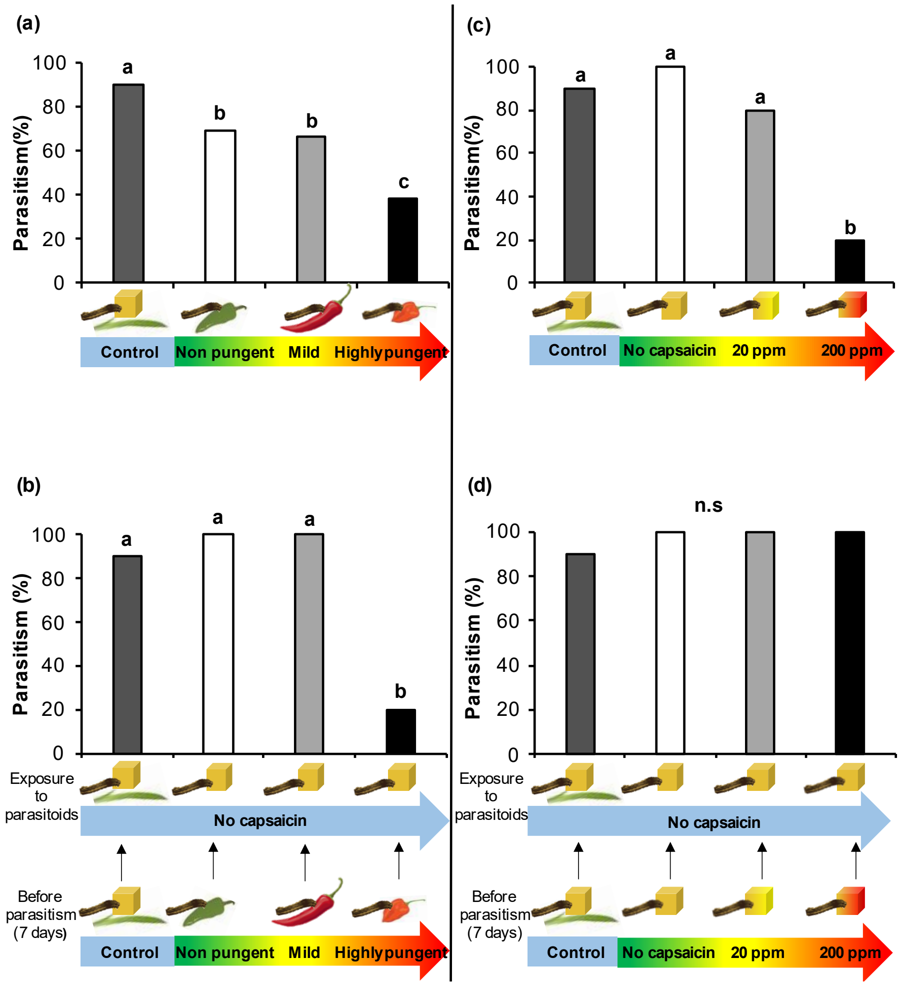


Fig. 3 Parasitism rate (%) of *Euplectrus platyhypenae* on *Spodoptera latifascia* caterpillars feeding on **a** a control diet and on chili fruits with three different pungency levels non-pungent, mild, and highly pungent, **b** on control diet and on chili fruits for 7 days and transferred to a regular artificial diet when exposed to the parasitoids, **c** a control diet and artificial diet mixed with three levels of synthetic capsaicin (no capsaicin, 20 and 200 ppm) and **d** a control diet and a capsaicin-spiked diet for 7 days before the parasitism and regular artificial diet when adding the parasitoids. For the control treatment, *S. latifascia* fed on maize leaf and regular artificial diet. Different letters indicate a significant difference between treatments (Chi-test, Tukey post hoc test with Bonferroni correction: $P < 0.01$). Sample sizes: Fig. 3a control = 10, non-pungent = 13, mild = 13 and highly pungent = 13; Fig. 3b, c and d, N = 5 for all treatments except for the control (N = 10).

2.3.4. Quantification of capsaicinoids in the haemolymph of *S. latifascia*

Capsaicinoids analysis in the haemolymph revealed that when caterpillars of *S. latifascia* were fed on habanero fruits, the levels of capsaicin and dihydrocapsaicin were 21 and 15 times, respectively, higher than when fed on fruits of the two other varieties (Fig. 4, capsaicin F[2,12] = 1.826, $P = 0.20$ and dihydrocapsaicin; F[2,12] = 1.3937, $P = 0.28$). Despite these very clear differences, the results were not significant most likely due to the high variability among larvae in the amount of placenta consumed, as capsaicinoids are mostly concentrated in this tissue and not in the rest of the fruit.

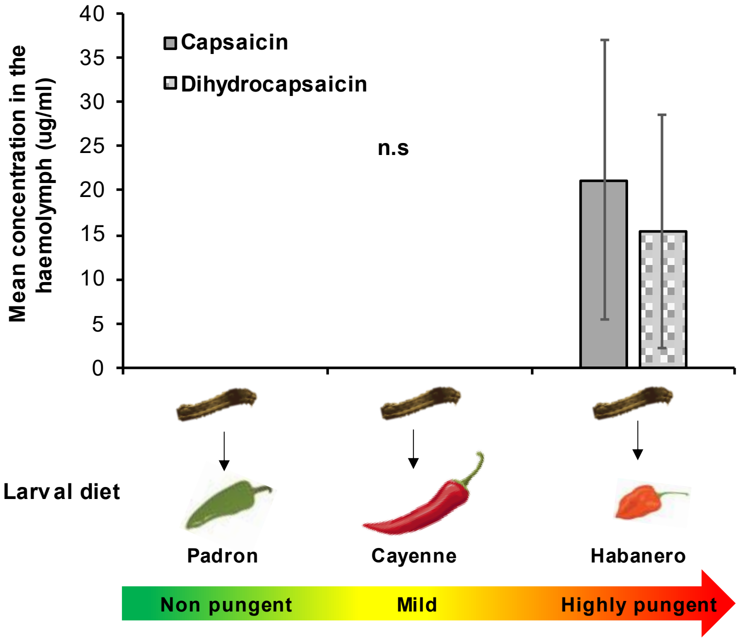


Fig. 4 Mean (± SEM) capsaicinoids content (mg/g of DW) in the haemolymph of *Spodoptera latifascia* larvae when feeding on three chili varieties, Padron, Cayenne and Habanero. (F-test: $P > 0.01$, N = 5)

2.4. Discussion

The aim of our study was to investigate the effect of capsaicin in domesticated chili peppers on a tritrophic interaction with the generalist herbivore and its ectoparasitoid *E. platyhypenae*. Overall, our results reveal that capsaicin had a negative effect on both insects, particularly at high concentrations. Indeed, we found that when larvae of *S. latifascia* were reared on pungent chili varieties, they had lower larval weight, reduced pupation, and lower adult emergence rates than when caterpillars were reared on non-pungent varieties. Similar results were found when caterpillars where reared on capsaicin-spiked and control diets. The negative effects of capsaicin subsequently affected the third trophic level by reducing the parasitism of caterpillars when these were reared on fruits or diet.

To date, all published studies on the effects of capsaicin on insects have focussed only on herbivores and most of these have used artificial diet or ground chili powder and not fresh fruits. These studies have mainly examined the benefits of capsaicin as a pesticide. For example, Cowles et al. (1989) found that oviposition of the onion fly (*Delia antiqua*) (Diptera: Anthomyiidae) was reduced by 99.8% and 95%, respectively, by applying both chili powder and synthetic capsaicin to artificial diet. Interestingly, despite capsaicinoids being naturally absent in leaves and vegetative organs of chili plants (Estrada et al. 2002), they are still effectively used as biopesticides against sucking insects attacking chili leaves, such as the green aphid *Myzus persicae* Sulz (Hemiptera: Aphididae) (Edelson et al. 2002; Koleva-Gudeva et al. 2013) or whiteflies, a major pest of pepper crops (Greer 2000). However, their mode of action remains unclear.

By using fresh chili fruits, we were able to assess the effect of natural capsaicin on the second and third trophic levels. Alternatively, the capsaicin-spiked diet allowed us to isolate the effect of capsaicin from other potential effects of the chili fruits on the insects. However, some differences were observed when using these two types of diets, particularly for the herbivore, where the negative effects of capsaicin were more evident when fed on fruits. We found that pungency level in fruits had a negative effect of on the parasitism both when the fruit was present or absent.

However, with diet this effect was only evident when the spiked-capsaicin diet was removed (Fig. 3d) as compared to when it was still present during parasitism (Fig. 3c). It is possible that due to a lower amount of capsaicin in diet as compared to fruits, the lasting effects inside the host were shorter and parasitoids were not able to perceive it. Due to the high capsaicin content in the fruits, we could not mimic the exact levels because of the high toxicity we experienced while manipulating the pure synthetic capsaicin. Therefore, we would expect that the effect would be stronger if we increase the capsaicin content levels equivalent to those found in the fruits.

It is expected that the effects of capsaicin will be different on generalist than specialist herbivores. For instance, larval growth of the generalist herbivores *Spodoptera frugiperda*, *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) was slower when fed with capsaicin-spiked diet, while the growth and survival of larvae of the tobacco budworm (*Helicoverpa assulta*) (Lepidoptera: Noctuidae), a specialist on Solanaceae, was not affected (Ahn et al. 2011a). The latter is considered to be one of the few insect herbivores capable of feeding on hot pepper fruits (Baek et al. 2009) and able to detoxify these secondary metabolites (Ahn et al. 2011b). Another species known to feed on chili pepper is the pepper weevil, *Anthonomus eugenii* (Coleoptera: Curculionidae), a specialist primarily on fruits of *Capsicum* spp., but able to feed on other nightshade plants (e.g. eggplants) (Rodriguez- Leyva 2006). This beetle is known to feed on highly pungent chili varieties such as Habanero and Scotch Bonnet (Seal and Martin 2016). Adults lay eggs on flower buds and complete their development inside the fruits (Riley and Sparks Jr 1995). Both larvae and adults were observed feeding on the fruit's placenta (Chabaane, personal observation) where capsaicinoids are concentrated (Fujiwake et al. 1982). It is assumed that both larvae and adults of this species can handle the spiciness, but the mechanism remains unclear. In our study, even though *S. latifascia* is a generalist, caterpillars were able to tolerate diet and fruits with medium levels of capsaicin. We found that while feeding, caterpillars can sequester capsaicin in the haemolymph, but only when they feed on the highly pungent varieties (Fig. 4). However, when exposed to lower levels of capsaicin, sequestration did not occur, or the levels were under the detection limit. It is possible that caterpillars are able to detoxify or excrete this secondary metabolite when present at low levels as it has been shown for other herbivores exposed to nicotine, also an alkaloid (Barbosa et al. 1986).

The effect of capsaicin on *S. latifascia* was stronger than on its parasitoid *E. platyhypenae*. For the parasitoid, we found differences among treatments only for parasitism rate but not for clutch size. Yet, as the parasitoid larvae feed on the host's haemolymph (Coudron et al. 1990; Nakamatsu and Tanaka 2003) where the capsaicin can be found, we could expect to find stronger effects of capsaicin on the parasitoid larval and adult stages. Thus, further studies should focus beyond the oviposition response of the parasitoid and examine the effects of capsaicin throughout parasitoid development, adult survival, size, and sex ratio.

Moreover, parasitism rate was also reduced when Habanero fruits were replaced by no-capsaicin diet during the exposure to wasps. This was probably caused by the capsaicinoids accumulated in the haemolymph. However, it remains to be investigated how long this accumulation will last, and the subsequent effects on the parasitoid once the exposure to capsaicin is stopped. It would also be interesting to test the effects of capsaicin on other natural enemies with different life history strategies and feeding modes such as endoparasitoids and predators.

Recently, a growing number of studies have examined the relationship between plant chemical defence as a result of domestication and insect performance (Chen et al. 2015; Whitehead et al. 2017). It is often found that lower chemical defence results in increased performance, but there are also many exceptions to this pattern (Shlichta et al. 2018; Turcotte et al. 2014). Chili pepper offers a unique model to examine this relationship, since we have varieties selected for lower capsaicinoid content than the wild chiltepin, but also varieties that were selected for much higher pungency levels (Scoville 1912). In another study, we found that the capsaicinoid levels in chiltepin fruits collected from different populations along the Pacific coast of the state of Oaxaca, Mexico, mainly ranged between the contents detected on Habanero and Cayenne varieties (Chabaane et al., unpublished results). Thus, we would expect that the performance of *S. latifascia* larvae on wild chili fruits would be intermediate compared to the highly pungent and mild varieties.

Here, we focussed on the effects of capsaicin, the main plant trait targeted during varietal selection of chili pepper (Paran and van der Knaap 2007). Yet, the domestication syndrome of Capsicum species includes other traits such as germination rate, fruit colour, position and size, foliar and phenological traits (Pickersgill 2016). It is likely that some or most of these traits will also affect insect choice and performance. Moreover, some of these traits might be correlated to the level of pungency in fruits. For example, Taiti et al. (2019) found that volatile organic compound (VOC) emissions from fresh chili fruits were correlated with their spiciness. It is known that parasitoids use plant volatiles to locate their hosts (Turlings et al. 1990; Vet and Dicke 1992). Therefore, future studies should investigate the influence of multiple domesticated traits on tritrophic interactions of this important crop.

Our results also offer further insight into alternative strategies for pest management in chili pepper, one of the top ten vegetable crops in the world (FAOSTAT 2020). The use of mixed varieties in agriculture has been shown to reduce pest pressure by slowing the spread of insects via resistant varieties acting as barriers (Barot 2017). For example, Abdala-Roberts et al. (2015) showed that mixed genotypes of *Capsicum chinense* reduced damage of the leaf mining fly (*Liriomyza trifolii*) (Diptera: Agromyzidae) by 25% as compared to monoculture. They suggest that plant genotypic diversity of their varieties (e.g. plant size, architecture, flowering phenology, and fruit size) played an important role in reducing insect attack. The importance of genotype diversity in chili peppers was also reported to decrease infestation by whitefly (*Bemisia tabaci*) (Hemiptera: Aleyrodidae) and yellow mite (*Polyphagotarsonemus latus*) (Acari: Tarsonemidae) (Datta and Chakraborty 2013), as well as black aphid (*Aphis craccivora*) (Homoptera: Aphididae) (Ofori et al. 2015) infestations on peppers. In this context, our results suggest that growing mixed chili varieties with different pungency levels, in addition to the use of parasitoids, might reduce pest pressure by generalist herbivores.

Therefore, chili pepper farmers should include these practices in their IPM (Integrated pest management) programmes to optimize pest control.

In conclusion, our study represents pioneering work regarding the effect of natural capsaicin on herbivores and the third trophic level. In the future, this knowledge could also be important for other crops, such as hot tomato, which could be developed by activating the inactive capsaicinoids biosynthesis pathway naturally present in this Solanaceous crop (Naves et al. 2019). Moreover, as chili peppers originate and were domesticated in Mesoamerica, by studying its interactions with native insects from this region, our results could provide insight into the selective pressures that have contributed to the crop's phenotypic diversity and the relationship with its wild ancestor.

Author contributions

YC and BB conceived and designed the research. YC performed experiments. YC and CMA developed the methods. YC and CMA analysed the data. GG performed the chemical analyses. YC and BB wrote the first drafts. All authors contributed to the last versions of the manuscript.

Acknowledgements

We thank Diane Laplanche and Gaia Besomi for helping with capsaicinoid extraction. This research was financially supported by a grant from the Swiss National Science Foundation (project no. 31003A-162860) awarded to Betty Benrey. The authors declare that they have no conflict of interest.

References

Abdala-Roberts L, Berny-Mier y Terán JC, Moreira X, Durán-Yáñez A, Tut-Pech F (2015) Effects of pepper (*Capsicum chinense*) genotypic diversity on insect herbivores. Agric For Entomol 17:433–438. https:// doi. org/ 10. 1111/ afe. 12125

Ahn S-J, Badenes-Pérez FR, Heckel DG (2011a) A host-plant specialist, *Helicoverpa assulta*, is more tolerant to capsaicin from *Capsicum annuum* than other noctuid species. J Insect Physiol 57:1212–1219. https:// doi. org/ 10. 1016/j. jinsp hys. 2011. 05. 015

Ahn SJ, Badenes-Pérez FR, Reichelt M, Svatoš A, Schneider B, Gershenzon J, Heckel DG (2011b) Metabolic detoxification of capsaicin by UDP-glycosyltransferase in three *Helicoverpa* species. Arch Insect Biochem Physiol 78:104–118. https:// doi. org/ 10. 1002/ arch. 20444

Argaez LGB, Salazar FFM, Zuñiga FZ, Estrada TG, Flores IRI (2009) Characterization of a *Capsicum chinense* seed peptide fraction with broad antibacterial activity. Asian J Biochem 4:77–87. https:// doi. org/ 10. 3923/ ajb. 2009. 77. 87

Aza-González C, Núñez-Palenius HG, Ochoa-Alejo N (2011) Molecular biology of capsaicinoid biosynthesis in chili pepper (*Capsicum* spp.). Plant Cell Rep 30:695–706. https:// doi. org/ 10. 1007/ s00299- 010- 0968-8

Baek S, Cho K, Song YH, Lee J-H (2009) Sampling plans for estimating pepper fruit damage levels by oriental tobacco budworm, *Helicoverpa assulta* (Guenee), in hot pepper fields. J Asia-Pac Entomol 12:175–178. https:// doi. org/ 10. 1016/j. aspen. 2009. 03. 003

Barbosa P, Saunders J, Kemper J, Trumbule R, Olechno J, Martinat P (1986) Plant allelochemicals and insect parasitoids effects of nicotine on *Cotesia congregate* (say) (Hymenoptera: Braconidae) and *Hyposoter annulipes* (Cresson)(Hymenoptera: Ichneumonidae). J Chem Ecol 12:1319–1328. https:// doi. org/ 10. 1007/ BF010 12351

Barot S et al (2017) Designing mixtures of varieties for multifunctional agriculture with the help of ecology. A Rev Agron Sustain Dev 37:13. https:// doi. org/ 10. 1007/ s13593- 017-0418-x

Benrey B, Denno RF (1997) The slow-growth–high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987–999. https:// doi. org/ 10. 1890/ 0012- 9658(1997) 078[0987: TSGHMH] 2.0. CO;2

Carrizo García C, Barfuss MH, Sehr EM, Barboza GE, Samuel R, Moscone EA, Ehrendorfer F (2016) Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae). Ann Bot 118:35–51. https:// doi. org/ 10. 1093/ aob/ mcw079

Chang’a A, de Souza N, Muya J, Keyyu J, Mwakatobe A, Malugu L, Ndossi HP, Konuche J, Omondi R, Mpinge A, Hahn N, Palminteri S, Olson D (2016) Scaling-up the use of chili fences for reducing human-elephant conflict across landscapes in Tanzania. Trop Conserv Sci 9:921–930. https:// doi. org/ 10. 1177/ 19400 82916 00900 220

Chen YH, Gols R, Benrey B (2015) Crop domestication and its impact on naturally selected trophic interactions. Annu Rev Entomol 60:35–58. https:// doi. org/ 10. 1146/ annur ev- ento-010814- 020601

Cichewicz RH, Thorpe PA (1996) The antimicrobial properties of chile peppers (*Capsicum* species) and their uses in mayan medicine. J Ethnopharmacol 52:61–70. https:// doi. org/ 10. 1016/0378- 8741(96) 01384-0

Collins MD, Wasmund LM, Bosland PW (1995) Improved method for quantifying capsaicinoids in *Capsicum* using high-performance liquid chromatography. HortScience 30:137–139. https://doi. org/ 10. 21273/ HORTS CI. 30.1. 137

Coudron TA, Kelly TJ, Puttler B (1990) Developmental responses of *Trichoplusia ni* (Lepidoptera: Noctuidae) to parasitism by the ectoparasite *Euplectrus plathypenae* (Hymenoptera: Eulophidae). Arch Insect Biochem Physiol 13:83–94. https:// doi. org/ 10. 1002/ arch. 94013 0108

Cowles R, Keller J, Miller J (1989) Pungent spices, ground red pepper, and synthetic capsaicin as onion fly ovipositional deterrents. J Chem Ecol 15:719–730. https:// doi. org/ 10. 1007/ BF010 14714

Cuny MA, Gendry J, Hernández-Cumplido J, Benrey B (2018) Changes in plant growth and seed production in wild lima bean in response to herbivory are attenuated by parasitoids. Oecologia 187:447–457. https:// doi. org/ 10. 1007/ s00442- 018- 4119-1

Datta S, Chakraborty G (2013) Studies on influence of genotypic diversity on yield, quality and incidence of white fly and yellow mite in *Capsicum annuum* L. J Appl Nat Sci 5:350–356. https:// doi. org/ 10. 31018/ jans. v5i2. 331

Edelson JV, Duthie J, Roberts W (2002) Toxicity of biorational insecticides: activity against the green peach aphid, *Myzus persicae* (Sulzer). Pest Manag Sci: Former Pestic Sci 58:255–260. https:// doi. org/ 10. 1002/ ps. 444

El-Heneidy A, Barbosa P, Gross P (1988) Influence of dietary nicotine on the fall armyworm, *Spodoptera frugiperda* and its parasitoid, the ichneumonid wasp *Hyposoter annulipes*. Entomol Exp Appl 46:227–232. https:// doi. org/ 10. 1111/j. 1570- 7458. 1988. tb011 16.x

Estrada B, Bernal MA, Díaz J, Pomar F, Merino F (2002) Capsaicinoids in vegetative organs of *Capsicum annuum* L. in relation to fruiting. J Agric Food Chem 50:1188–1191. https://doi. org/ 10.1021/ jf011 270j

FAOSTAT (2020) Global production of vegetables in 2018, by type. https:// www. statista. com/ statistics/ 264065/ global- production- of vegetables- by- type/. Accessed 12 January 2021

Fujiwake H, Suzuki T, Iwai K (1982) Capsaicinoid formation in the protoplast from the placenta of *Capsicum* fruits. Agric Biol Chem 46:2591–2592. https:// doi. org/ 10. 1080/ 00021 369. 1982. 10865 477

Gepts P (2004) Crop domestication as a long-term selection experiment. Plant Breed Rev 24:1–44. https:// doi. org/ 10. 1002/ 97804 70650 288

Godfray HCJ (1994) Taxonomic Index. In: Parasitoids, vol 67. Behavioral and Evolutionary Ecology. Princeton University Press, pp 465–473. https:// doi. org/ 10. 2307/j. ctvs3 2rmp

González-Zamora A, Sierra-Campos E, Pérez-Morales R, Vázquez-Vázquez C, Gallegos-Robles MA, López-Martínez JD, García-Hernández JL (2015) Measurement of capsaicinoids in chiltepin hot pepper: a comparison study between spectrophotometric method and high performance liquid chromatography analysis. J Chem 2015:10. https:// doi. org/ 10. 1155/ 2015/ 709150

Govindarajan V, Salzer UJ (1985) *Capsicum*-production, technology, chemistry, and quality part 1: history, botany, cultivation, and primary processing. Crit Rev Food Sci Nutr 22:109 176. https:// doi.org/ 10. 1080/ 10408 39850 95274 12

Greer L (2000) Greenhouse IPM: sustainable whitefly control. The National Center for Appropriate Technology (NCAT), Butte. http:// micha eljbo we. com/ TSG/ SG/ SF_ 00256. pdf
Haak DC, McGinnis LA, Levey DJ, Tewksbury JJ (2012) Why are not all chilies hot? A trade-off limits pungency. Proc R Soc B: Biol Sci 279:2012–2017. https:// doi. org/ 10. 1098/rspb. 2011. 2091

Habib M, Paleari L, Amaral M (1982) Effect of three larval diets on the development of the armyworm, Spodoptera latifascia Walker, 1856 (Noctuidae, Lepidoptera). Rev Bras De Zool 1:177–182. https:// doi. org/ 10. 1590/ S0101- 81751 98200 03000 07

Haine ER, Rolff J, Siva-Jothy MT (2007) Functional consequences of blood clotting in insects. Dev Comp Immunol 31:456–464. https://doi. org/ 10. 1016/j. dci. 2006. 08. 004

Hedges S, Gunaryadi D (2010) Reducing human–elephant conflict: do chillies help deter elephants from entering crop fields? Oryx 44:139–146. https:// doi. org/ 10. 1017/ S0030 60530 99900 93

Hori M, Nakamura H, Fujii Y, Suzuki Y, Matsuda K (2011) Chemicals affecting the feeding preference of the solanaceae-feeding lady beetle *Henosepilachna vigintioctomaculata* (Coleoptera: Coccinellidae). J Appl Entomol 135:121–131. https:// doi. org/ 10. 1111/j. 1439-0418. 2010. 01519.x

Jones NL, Shabib S, Sherman PM (1997) Capsaicin as an inhibitor of the growth of the gastric pathogen *Helicobacter pylori*. FEMS Microbiol Lett 146:223–227. https:// doi. org/ 10. 1111/j. 1574- 6968. 1997. tb101 97.x

Jordt S-E, Julius D (2002) Molecular basis for species-specific sensitivity to “hot” chili peppers. Cell 108:421–430. https:// doi. org/ 10. 1016/ S0092- 8674(02) 00637-2

Katz E (2009) Chili pepper, from Mexico to Europe. Food, imaginary and cultural identity. Food, Imaginaries and Cultural Frontiers Essays in honour of Helen Macbeth, Guadalajara, Universidad de Guadalajara, Colección Estudios del Hombre, Serie Antropología de la Alimentación:213–232

Kim S et al (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. Nat Genet 46:270–278. https:// doi. org/ 10. 1038/ ng. 2877

Saunders JL, Coto D, King ABS (1998) Plagas invertebradas de cultivos anuales alimenticios en América Central, 2da edición. Centro Agronómico Tropical de Investigación y Enseñanza, CATIE 29:305. http:// repos itorio. bibli oteca orton. catie. ac. cr/ handle/ 11554/ 3346

Koleva-Gudeva L, Mitrev S, Maksimova V, Spasov D (2013) Content of capsaicin extracted from hot pepper (*Capsicum annuum* ssp. microcarpum L.) and its use as an ecopesticide. *Hemijska Industrija* 67:671–675. https:// doi. org/ 10. 2298/ HEMIN D1209 21110K

Ladizinsky G (2012) Plant evolution under domestication. Springer Science & Business Media, Berlin. https:// doi. org/ 10. 1007/978- 94- 011- 4429-2

Lang Y, Yanagawa S, Sasanuma T, Sasakuma T (2006) A gene encoding a putative acyl-transferase involved in pungency of *Capsicum*. Breed Sci 56:55–62. https:// doi. org/ 10. 1270/ jsbbs. 56. 55

Marini E, Magi G, Mingoia M, Pugnaloni A, Facinelli B (2015) Antimicrobial and anti-virulence activity of capsaicin against erythromycin-resistant, cell-invasive group a streptococci. Front Microbiol 6:1281. https:// doi. org/ 10. 3389/ fmicb. 2015. 01281

Mason JR, Maruniak J (1983) Behavioral and physiological effects of capsaicin in red-winged blackbirds. Pharmacol Biochem Behav 19:857–862. https:// doi. org/ 10. 1016/ 0091- 3057(83) 90093-X

Meyer RS, DuVal AE, Jensen HR (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. New Phytol 196:29–48. https:// doi. org/ 10. 1111/j. 1469- 8137. 2012. 04253.x

Molina Torres J, García Chávez A, Ramírez Chávez E (1999) Antimicrobial properties of alkamides present in flavouring plants traditionally used in mesoamerica: affinin and capsaicin. J Ethnopharmacol 64:241–248. https:// doi. org/ 10. 1016/ s0378- 8741(98) 00134-2

Muniappan R, Bamba J, Cruz J, Reddy G (2004) Biology, rearing and field release on guam of *Euplectrus maternus*, a parasitoid of the fruit-piercing moth, *Eudocima fullonia*. Biocontrol 49:537–551. https:// doi. org/ 10. 1023/B: BICO. 00000 36439. 74117. 2f

Muñoz-Ramírez LS, Peña-Yam LP, Avilés-Viñas SA, Canto-Flick A, Guzmán-Antonio AA, Santana-Buzzy N (2018) Behavior of the hottest chili peppers in the world cultivated in Yucatan, Mexico. HortScience 53:1772–1775. https:// doi. org/ 10. 21273/ HORTS CI135 74-18
Murúa G, Virla EG (2004) Contribution to the biological knowledge of *Euplectrus platyhypenae* (Hymenoptera: Eulophidae), a parasitoid of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Argentina. Folia Entomológica Mexicana 43:171–180

Nakamatsu Y, Tanaka T (2003) Venom of ectoparasitoid, *Euplectrus* sp. near *plathypenae* (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. J Insect Physiol 49:149–159. https:// doi. org/ 10. 1016/ S0022- 1910(02) 00261-5

Nakamatsu Y, Tanaka T (2004) Venom of *Euplectrus separatae* causes hyperlipidemia by lysis of host fat body cells. J Insect Physiol 50:267–275. https:// doi. org/ 10. 1016/j. jinsp hys. 2003. 12. 005

Naves ER, de Ávila SL, Sulpice R, Araújo WL, Nunes-Nesi A, Peres LE, Zsögön A (2019) Capsaicinoids: pungency beyond *Capsicum*. Trends Plant Sci 24:109–120. https:// doi. org/ 10. 1016/j. t plants. 2018. 11. 001

Ofori ESK, Appiah AS, Nunekpeku W, Quartey EK, Owusu-Ansah M, Amoatey HM (2015) Relative abundance and diversity of insect species on nine genotypes of pepper (Capsicum spp.) grown under field conditions in Ghana. J Exp Agric Int. https:// doi. org/ 10. 9734/ AJEA/ 2015/ 12150

Omolo MA et al (2014) Antimicrobial properties of chili peppers. J Infect Dis Ther 2:145. https:// doi. org/ 10. 4172/ 2332- 0877. 1000145

Opitz SE, Müller C (2009) Plant chemistry and insect sequestration. Chemoecology 19:117–154. https:// doi. org/ 10. 1007/ s00049- 009- 0018-6

Paran I, van der Knaap E (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. J Exp Bot 58:3841–3852. https:// doi. org/ 10. 1093/ jxb/ erm257

Pickersgill B (2016) Chile Peppers (Capsicum spp.). In: Lira R, Casas A, Blancas J (eds) Ethnobotany of Mexico. Ethnobiology. Springer, New York, NY. https:// doi. org/ 10. 1007/ 978-1- 4614-6669-7_ 17

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annu Rev Ecol Syst 11:41–65. https://d oi. org/ 10. 1146/ annur ev. es. 11. 110180. 000353
Riley D, Sparks AN (1995) The pepper weevil and its management. AgriLife Extension, L-5069. Texas A & M University, Bryon- College Station, TX. Available electronically from https:// hdl. handle. net/ 1969.1/ 87688

Rodriguez-Leyva E (2006) Life history of Triaspis eugenii Wharton and López-Martínez (Hymenoptera: Braconidae) and evaluation of its potential for biological control of pepper weevil *Anthonomus eugenii* Cano (Coleoptera: Curculionidae). Dissertation, University of Florida,

Rowell-Rahier M, Pasteels JM (1992) Chapter 6 - Third Trophic Level Influences of Plant Allelochemicals. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites (Second Edition). Academic Press, San Diego, pp 243–277. https:// doi. org/ 10. 1016/ B978-0- 08- 092545- 5. 50011-0

Schaffner U, Boevé J-L, Gfeller H, Schlunegger UP (1994) Sequestration of Veratrum alkaloids by specialist *Rhadinoceraea nodicornis Konow* (Hymenoptera, Tenthredinidae) and its ecoethological implications. J Chem Ecol 20:3233–3250. https:// doi. org/ 10. 1007/ BF020 33723

Scoville WL (1912) Note on capsicums. J Am Pharm Assoc 1:453–454. https:// doi. org/ 10. 1002/ jps. 30800 10520

Seal DR, Martin CG (2016) Pepper weevil (Coleoptera: Curculionidae) preferences for specific pepper cultivars, plant parts, fruit colors, fruit sizes, and timing. Insects 7:9. https:// doi. org/ 10. 3390/ insects701 0009

Shlichta JG, Cuny MA, Hernandez-Cumplido J, Traine J, Benrey B (2018) Contrasting consequences of plant domestication for the chemical defenses of leaves and seeds in lima bean plants. Basic Appl Ecol 31:10–20. https:// doi. org/ 10. 1016/j. baae. 2018. 05. 012

Smartt J, Simmonds NW (1995) Evolution of crop plants (No. Sirsi) i9780470233726)

Stewart C Jr et al (2005) The pun1 gene for pungency in pepper encodes a putative acyltransferase. Plant J 42:675–688. https:// doi. org/ 10. 1111/j. 1365- 313X. 2005. 02410.x

Swezey O (1924) The Mexican armyworm parasite (*Euplectrus platyhypenae*).
Hawaii Plant Rec 28:318–320

Szolcsányi J, Sann H, Pierau F-K (1986) Nociception in pigeons is not impaired by capsaicin. Pain 27:247–260. https:// doi. org/ 10. 1016/ 0304- 3959(86) 90215-0

Taiti C, Costa C, Migliori CA, Comparini D, Figorilli S, Mancuso S (2019) Correlation between volatile compounds and spiciness in domesticated and wild fresh chili peppers. Food Bioprocess Technol 12:1366–1380. https:// doi. org/ 10. 1007/ s11947- 019- 02297-9
Tęgowska E, Grajpel B, Piechowicz B (2005) Does red pepper contain an insecticidal compound for colorado beetle? IOBC Wprs Bull 28:121–127

Tewksbury JJ, Nabhan GP (2001) Directed deterrence by capsaicin in chillies. Nature 412:403–404. https:// doi. org/ 10. 1038/ 35086 653

Tewksbury JJ, Reagan KM, Machnicki NJ, Carlo TA, Haak DC, Peñaloza ALC, Levey DJ (2008) Evolutionary ecology of pungency in wild chilies. Proc Natl Acad Sci 105:11808–11811. https:// doi. org/ 10. 1073/ pnas. 08026 91105

Traine J, Cuny MA, Bustos-Segura C, Benrey B (2020) The interaction between host and host plant influences the oviposition and performance of a generalist ectoparasitoid. Entomol Exp Appl 169:133–144. https:// doi. org/ 10. 1111/ eea. 12949

Tsurumaki K, Sasanuma T (2019) Discovery of novel unfunctional pAMT allele pamt10 causing loss of pungency in sweet bell pepper (*Capsicum annuum* L.). Breed Sci 69:133–142. https:// doi.org/ 10. 1270/ jsbbs. 18150

Turcotte MM, Turley NE, Johnson MT (2014) The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. New Phytol 204:671 681. https://doi. org/ 10. 1111/ nph. 12935

Turlings TC, Benrey B (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. Ecoscience 5:321–333. https:// doi. org/ 10. 1080/ 11956 860. 1998. 11682 472

Turlings TC, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251–1253. https:// doi. org/ 10. 1126/ science. 250. 4985. 1251

Vet LE, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annu Rev Entomol 37:141–172. https:// doi. org/ 10. 1146/ annur ev. en. 37. 010192. 001041

Weissenberg M, Klein M, Meisner J, Ascher K (1986) Larval growth inhibition of the spiny bollworm, Earias insulana, by some steroidal secondary plant compounds. Entomol Exp Appl 42:213–217. https:// doi. org/ 10. 1111/j. 1570- 7458. 1986. tb010 24.x

Whitehead SR, Turcotte MM, Poveda K (2017) Domestication impacts on plant–herbivore interactions: a meta-analysis. Philos Trans R Soc B: Biolo Sci 372:20160034. https:// doi. org/ 10. 1098/ rstb.2016. 0034

Wilson W (1996) Wax and capsaicin based pesticide. J Clean Prod 1:61

Witting N, Svensson P, Gottrup H, Arendt-Nielsen L, Jensen TS (2000) Intramuscular and intradermal injection of capsaicin: a comparison of local and referred pain. PAIN® 84:407–412. https:// doi. org/ 10. 1016/ S0304- 3959(99) 00231-6

Zagatti P, Lalanne-Cassou B, le Duchat d’Aubigny J (1995) Catalogue of the lepidoptera of the French antilles. INRA. Accessed from INRA Database. http:// www7. inra. fr/ papil lon/ index eng. htm

Supplementary material

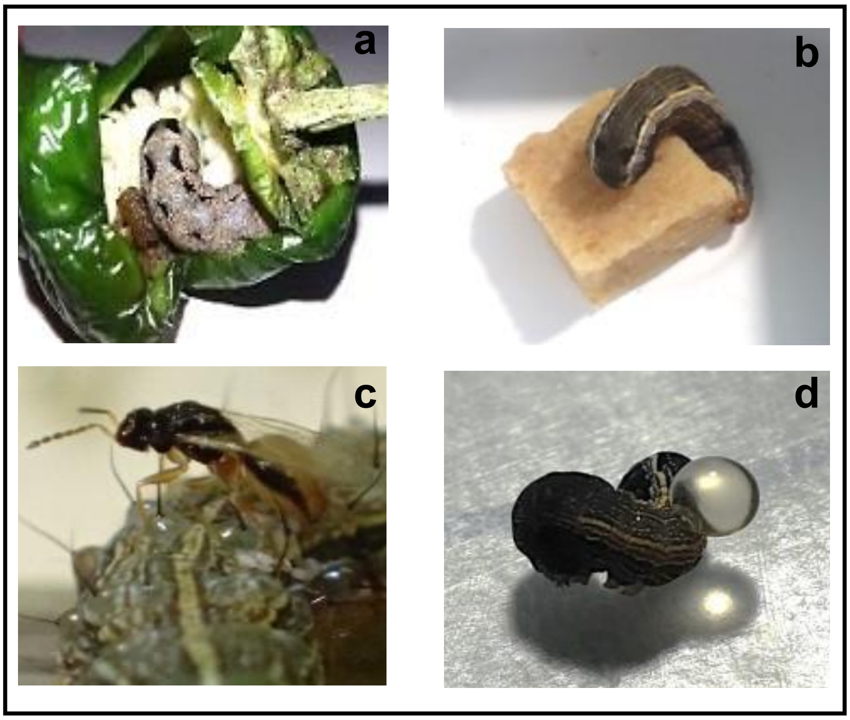


Fig. S1 *Spodoptera latifascia* caterpillars feeding on chili fruits **a**, on artificial diet **b**, its female parasitoid *Euplecterus platyhypenae* **c** and its haemolymph extracted from the dorsal part of the thorax **d.**

Table S1. Mean pupal weight (mg) of *Spodoptera latifascia* feeding on chili varieties with three different pungency levels: non pungent (Padron), mild (Cayenne) and highly pungent (Habanero) (F-test: P > 0.05, Sample sizes = Number of pupa)

Pungency level	Variety name	Mean pupal weight (mg)	Standard Errors	Number of pupa
Non pungent	Padron	542.81	14.37	15
Mild	Cayenne	568,43	44.49	4
Highly pungent	Habanero	539,16	74.16	5

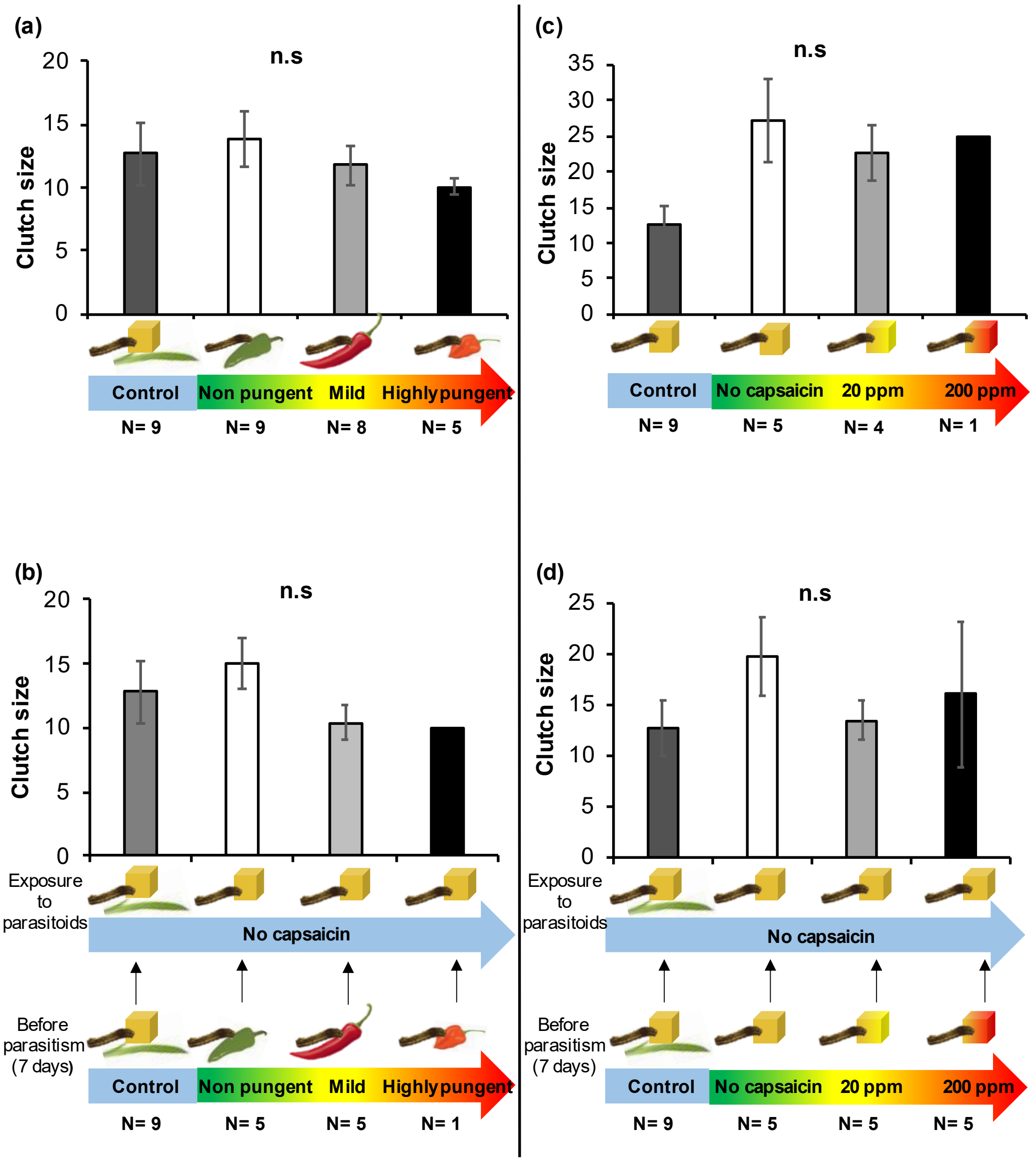


Fig. S2 Clutch size (Mean (±SEM) number of parasitoid eggs laid on a caterpillar) of *Euplectrus platyhypenae* on *Spodoptora latifascia* caterpillars feeding on **(a)** control diet and on chili fruits with three different pungency levels non pungent, mild, and highly pungent, **(b)** on control diet and on chili fruits for 7 days and transferred to a regular artificial diet when exposed to the parasitoids, **(c)** on control diet and artificial diet mixed with three levels of synthetic capsaicin (no capsaicin, 20 and 200 ppm) and **(d)** on control diet and a capsaicin-spiked diet for 7 days before the parasitism and regular artificial diet when adding the parasitoids. For the control treatment, *S.latifascia* fed on maize leaf and regular artificial diet. No significant difference between treatments (F-test: $P > 0.05$). Sample sizes are indicated directly in the figures (N = number of parasitized larva)

3. Domestication of chili pepper has increased susceptibility to herbivore damage and whitefly-transmitted virus in the field

Yosra Chabaane[1], Raul Gonzalez[2], Elsa Kumar[1], and Betty Benrey[1*]

[1]Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

[2]Laboratory of Ecological Interactions and Processes, Department of Ecology and Natural Resources, National Autonomous University of Mexico, Coyoacan delegation, 04510 Mexico City, Mexico. rulobio@ciencias.unam.mx

[*]Author for correspondence (e-mail: betty.benrey@unine.ch)

Abstract

Plant domestication aims to reduce plant defenses and select varieties with a higher yield than their wild ancestors. It is believed that these altered traits have an impact on plant-insect interactions. However, in the case of chili pepper, these effects remain unclear.

In a common garden experiment, wild chilies were more resistant to chewing insects and virus infection but not for their vector, whitefly. Moreover, chili domestication has altered trichome density and phenolic compounds in leaves. On the one hand, domestication decreased trichome density that could play a role in plant resistance against chewing insects. On the other hand, domesticated plants had more phenolic compounds in leaves than wild chilies, suggesting that these compounds play different roles in the plant, such as protecting chili varieties from potential stress caused by UV radiation. Interestingly, birds selected only fruits from wild accessions. They play an essential role in chili domestication by selecting varieties protected from seed dispersal. This work is a pioneering study of all these natural interactions with chili pepper in the region where wild and cultivated plants coexist.

Conducting fieldwork in the center of domestication of chili pepper was a crucial step to report all these multitrophic interactions. However, further work both in situ and in the laboratory is needed to understand these complex interactions better.

Key Words: Chili peppers, domestication, whiteflies, virus, birds, natural habitat.

3.1. Introduction

Crop domestication plays a pivotal role in assuring food security in the world. It is defined as the artificial selection of plant traits to make them more suitable for human taste and better adapted for cultivation (Ladizinsky 2012). As a result, domesticated plants differ from their wild relatives in morphological traits, chemistry, and nutritional content (Gepts 2004; Meyer et al. 2012). In recent years, there has been an increased interest in elucidating the effects of altered plant traits due to domestication on the insects that attack crop plants (see reviews by (Chen et al. 2015; Fernandez et al. 2021; Whitehead et al. 2017). Overall, studies show that selection for lower toxicity and increased nutrient content in crops compared to their wild counterparts has improved herbivore performance (Benrey et al. 1998; Dávila-Flores et al. 2013; Gols et al. 2008; Idris and Grafius 1996; Rodriguez-Saona et al. 2019). However, most of these studies have been conducted in laboratory or greenhouse settings, and comparable evidence from field studies examining domesticated and wild plants' susceptibility to herbivore attack is scarce (Chen and Welter 2002; Urbaneja-Bernat et al. 2020). This knowledge could be fundamental in domestication centers where crops still coexist with their wild relatives and where local biotic and abiotic factors and human preferences have likely contributed to the selection of plant phenotypes (Chen et al. 2017).

Chili pepper is among the most grown plants globally (Andrews 1995; FAOSTAT 2020) and has become a crucial ingredient in many countries' cuisines. Although chili pepper was not an essential crop before the European conquest, its domestication dates back to 10 000 years (Aguilar-Meléndez et al. 2009). Based on molecular analyses of several domesticated and wild species, Walsh and Hoot (2001) suggest that the genus *Capsicum* most likely originated from the Andean mountains, then reached lowland regions in the Americas. This genus includes around 35 species (Carrizo García et al. 2013), from which five were domesticated in different parts of Mexico, Central and South America (Pickersgill 1997). The *Capsicum annuum* complex containing the three species *C. annuum*, *C. chinense*, *C. frutescens* that can cross-pollinate is economically the most important worldwide (Pereira-Dias et al. 2019). *Capsicum baccatum* and *C. pubescens* are mainly cultivated in South America. Each species derived from a different wild ancestor (Bosland et al. 2012). The wild chiltepin (*C. annuum var. glabriusculum*) is most likely the ancestor of *C. annuum* and was domesticated in Mexico (Kraft et al. 2014).

The suite of traits that distinguish a crop plant from its wild relative is known as the domestication syndrome (Hammer 1984). For chili pepper, this includes an increase in seed germination rate and altered fruit traits such as an increase in fruit size and changes in the fruit position from upright to pendant hidden by the foliage where the fruit is more protected from bird dispersal and remains attached to the plant when fully ripe (Pickersgill 2016a). However, the germination rate is the only consistent trait-distinguishing wild from domesticated chilies with rapid uniform germination necessary for producing higher yields (Pickersgill 2007). After the initial domestication phase, varieties of chili pepper were further selected for other traits such as the degree of pungency, color, and thickness of the fruit (Ladizinsky 2012). Altered leaf traits are poorly reported for chili, only larger leaves in domesticated plants compared to wild (Pickersgill 2016). Despite their worldwide economic importance, the extent to which domesticated traits have influenced the interaction between chili plants and their associated herbivorous pests is poorly known.

Different herbivores have been reported to attack chili pepper plants such as aphids, armyworms, tobacco and tomato hornworms, and pepper weevils (Daryanto et al. 2020; Garvey et al. 2020; Ozores-Hampton et al. 2014; Seal and Martin 2016). One of the most destructive pests of chili peppers is the sweet potato whitefly (SPW), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Ballina-Gomez et al. 2013; Nasruddin et al. 2020). It is considered a cryptic species complex, containing at least 24 morphologically indistinguishable species and many different populations called “biotypes” (Barro et al. 2011; Perring 2001). During field surveys conducted in Puerto Escondido (Oaxaca, Mexico) in 2019 and 2020, farmers confirmed that whiteflies and mainly the virus transmitted by them, account for the principal cause of damage in their chili fields (Chabaane, unpublished data). Whiteflies can cause damage to plants in three different ways: (1) suck the sap on leaves with their stylus, (2) produce honeydew, facilitating the infection by pathogenic fungi and bacteria, and (3) transmit different plant viruses (Basu 2019). In the case of chili pepper, *Bemisia tabaci* is known to transmit a wide variety of Begomoviruses causing several diseases such as the pepper yellow leaf curl disease or yellow rugosing disease known in Mexico as the "*rizado amarillo*" (De Barro et al. 2008; Maruthi et al. 2007; Morales and Anderson 2001; Padhi et al. 2017). The typical symptoms of virus infection are vein yellowing, foliar yellow mosaics, and leaf curling (Hamilton et al. 1981). Begomoviruses are exclusively transmitted by whiteflies, principally in a persistent, circulative, and non-propagative manner (Rosen et al. 2015). To cope with the potential risk of the complex of *B. tabaci*-virus, farmers spray chemical insecticides two to three times per week, and this has led to highly resistant populations of *B. tabaci* (Elbert and Nauen 2000). Therefore, increasing host plant resistance against whiteflies might be a more effective and sustainable solution for crop management. In this context, plant domestication studies that compare the susceptibility of wild and domesticated plants to their natural enemies can identify plant traits responsible for the plant's natural resistance against pests (Sharma and Ortiz 2002). These traits could then be favored in plant breeding programs.

Several studies have examined the physical and chemical plant traits that enhance plant resistance against *B. tabaci*. Among these, glandular trichomes, leaf shape, and cuticle thickness (Berlinger 1986; Hasanuzzaman et al. 2016; Oriani and Vendramim 2010). In addition, the chemical composition of leaf sap, nutritional compounds of leaves, and plant volatiles have also been implicated in resistance (Chen et al. 2021; Hasanuzzaman et al. 2017). The relationship between plant traits and resistance against *B. tabaci* has been well studied for several crops, including tomato, eggplant, vigna, and cassava, both in the field and greenhouses (Bellotti and Arias 2001; Chen et al. 2021; Taggar and Gill 2016; Taher et al. 2020). For chili pepper, (Ballina-Gomez et al. 2013) conducted a morphological characterization of 18 wild and semiwild accessions of *C. annum* from southern Mexico and evaluated their response to the complex *B. tabaci*-begomoviruses. Only tillering and plant canopy width were negatively correlated with disease severity, but no relationship was found with virus incidence and density of whitefly populations.

This study aimed to examine the effects of chili domestication on plant susceptibility to herbivore attack in the field. Based on the high densities of whiteflies observed in previous years and on farmers' accounts, we mainly focused on infestation by whiteflies and the virus transmitted by them. We hypothesized that domesticated varieties are more susceptible to whitefly attack and virus infestation than wild accessions. To test this hypothesis, we set up a common garden experiment with three wild accessions and three domesticated chili varieties in an open field during two successive seasons, 2019 and 2020. Plants were followed throughout the season, and we recorded whitefly infestation and virus infection. In addition, we recorded herbivore damage by chewing insects and the number of leaves and fruits produced per plant. Two types of defenses in leaves were measured: Trichome density (physical defense) and phenolic content (chemical defense). In addition, we sampled nine populations of wild chilies around Puerto Escondido (Oaxaca, Mexico) to characterize their fruits according to the pungency levels and check for herbivore damage and symptoms of virus infection. This information is important to evaluate furthermore the resistance of wild chiltepin accessions in their natural habitats.

Our specific objectives were to (1) examine whether domestication has altered physical and chemical defense traits in leaves, and (2) investigate whether these altered traits have affected the susceptibility of wild and domesticated plants to herbivory and whitefly-transmitted virus. Conducting this study in Mexico, the center of domestication of chili pepper could shed some light on how biotic and abiotic factors have affected varietal selection and diversification. Finally, a better knowledge of the natural resistance of wild plants could help develop new varieties and improve existing ones.

3.2. Material and methods

3.2.1. Study site

Field experiments were conducted at 15 km North-West of Puerto Escondido (Oaxaca, Mexico, 15°55'33.3" N 97°09'03.0" W). We carried out two field experiments, from November 2018 to February 2019, "2019 experiment" hereafter and from December 2019 to February 2020, "2020 experiment" hereafter.

In Puerto Escondido, wild chili called "Chile Piquin" or "Chiltepin" is found in backyard gardens with other domesticated varieties or grown naturally in the forest (Figure S1). It is harvested by locals and sold in markets, as it is a vital ingredient in their local cuisine. In addition, chili pepper is often planted with other crops, for example, in the intercrop-growing system known as Milpa, together with beans, maize, and squash (Aragón-Cuevas et al. 2004). Moreover, the use of mixed local chili varieties (eg. Tusta and Costenõ) are common in the region (Chabaane, personal observation).

3.2.2. Leaf defense measurements: trichome density and phenolic content

- **Trichome density**

To estimate trichome density on leaves, we cut 1 cm^2 in the central part and 1 cm^2 on the leaf edge. For each variety and wild accession, we randomly chose four plants per treatment from the common garden. From each plant, we selected three leaves, one from the top, one from the middle, and one from the bottom. The trichomes on the upper, lower, and edge parts were counted with a microscope (MOTIC BA-310). We used twelve samples from each variety/accession (N=12 leaves, three leaves per plant, and four plants per treatment).

- **Phenolic content**

As an indicator of chemical defense in leaves against insect herbivores, we quantified the content of phenolic compounds. For extraction, we used 200 mg of grounded fresh leaves diluted in 2 ml of aqueous methanol (1:1 vol) following the method used by Moreira et al. (2014). Then, samples were placed in an ultrasonic bath for 15 min for cleaning and sterilization, followed by centrifugation for 2 min (10000 rpm). After, we took 300 ul of supernatant and diluted it in 100 ul of water. Following the extraction, the samples were analyzed with an Ultra-High-Pressure Liquid Chromatography-Quadrupole-Time-Of-Fight Mass Spectrometry (UHPLC-QTOF-MS) to detect, identify, and quantify phenolic compounds.

3.2.3. Insect damage on wild and domesticated chili plants in a common garden experiment

Seeds of wild and domesticated plants were individually planted in biodegradable round fiber pots (6 × 96cm). All plants were kept in a field tent (Bioquip Outdoor Cage 6’×6’×6', 20×20 Mesh Lumite) to protect them from herbivores until they had developed 6 to 8 leaves, at which time they were transplanted to a common garden. Plants were watered every other day. We used three domesticated varieties and three wild accessions as referred to in Table S1. They are all *Capsicum annuum* species except for the Habanero variety, which is *Capsicum Chinense,* known to be more pungent than the wild Chiltepin (*Capsicum annuum var. glabriusculum*) according to the Scoville scale (Scoville 1912). This variety was selected in the Yucatan Peninsula in Mexico (Muñoz-Ramírez et al. 2018) and is widely used in Oaxaca, readily found in local markets (Marina Clemente et al. 2020). The wild accessions were obtained from native seeds (www.nativeseeds.org, accessed on 10 July 2021), and they are originally from Mexico.

- **Experimental design**

The common garden array was established in twelve blocks. Each block consisted of six subplots representing the three domesticated varieties and the three wild accessions randomly arranged in two rows, as illustrated in Figure S2. For each subplot, we planted three to two plants from the same variety/accession. Thus, the total number of plants used for the common garden was 216 for the 2019 experiment and 144 for the 2020 experiment following the same experimental design. Blocks were 1.5 m apart, and the distance of subplots within rows was 1m. The plants' position was randomized differently for the 2019 and 2020 experiments.

Monitoring was done once a week from 7 am to 11 am and lasted eight and seven weeks for the 2019 and 2020 experiments, respectively. Every week, the following parameters were recorded: total leaf number, leaf area damaged, number and insect identity according to three categories: chewing insects, sucking insects (whiteflies excluded), natural enemies, the total number of fruits produced at the end of the season, and the number of damaged fruits. From the beginning of the season, it became clear that whiteflies were the most abundant herbivores observed on the plants; we gave special attention to these and recorded the presence of whiteflies and virus infection for each plant. At the time of our experiments, the field site was also used for parallel experiments with squash, maize and milpa experimental plots with maize, beans and squash plants. In this site, we could find natural populations of herbivorous insects that readily feed on chili plants, including lepidopterans (*Spodoptera latifascia* and the less common *Manduca* spp), chrysomelid beetles (e.g., *Diabrotica balteata*) (Table S2), in addition to whiteflies.

- **Plant growth: number of leaves**

We recorded the total leaf number for domesticated varieties and wild accessions during the 2019 and 2020 experiments to follow the plants' growth. Per plant, each fully expanded leaf was counted once a week.

- **Chewing insect attack on leaves**

We visually estimated the area removed from each leaf and scored the damage according to 4 categories of leaf area removed (0-25%, 25%-50%, 50%-75%, and 75%-100%). To calculate the total area removed (**TAR**) by the herbivore per plant, we used the midpoint of each category following the formula below (Abdala-Roberts et al. 2015):

- **TAR= (PLD_{25}*12,5+ PLD_{50}*37,5+ PLD_{75}*62,5+PLD_{100}*87,5)/100**
- **PLD_{25}**: Percentage of leaves damaged in the category between 0 to 25 %.
- **PLD_{50}**: Percentage of leaves damaged in the category between 25 to 50 %.
- **PLD_{75}**: Percentage of leaves damaged in the category between 50 to 75 %.
- **PLD_{100}**: Percentage of leaves damaged in the category between 75 to 100 %.

% of damaged leaves per plant = (number of damaged leaves/number of total leaves)*100

The leaf area removed represents attacks by chewing insects and mainly the velvet armyworm *Spodoptera latifascia* commonly present in this site (Bustos-Segura et al. 2020; Traine et al. 2020).

- **Whitefly infestation and virus infection on leaves**

For the whiteflies, it was not possible to count the number of adults per plant as they are too tiny and fly quickly as soon as we approach the plant. Therefore, the whitefly infestation was quantified directly by the proportion of infested plants (= plants on which we observed this insect/total number of plants) and indirectly by looking at virus symptoms on leaves caused by whiteflies (Figure S3).

We recorded the proportion of virus-infested plants to evaluate the virus incidence (proportion of virus-infested plants = number of plants with virus symptoms / total number of plants). Moreover, the intensity or level of infection that was recorded the last week of both experiments (2019 and 2020) as follows:

- ➢ No infection: plants without virus symptoms.
- ➢ Low infection: less than 25 % of total leaves having virus symptoms.
- ➢ Medium infection: between 25 % and 75 % of total leaves having virus symptoms.
- ➢ High infection: more than 75 % of total leaves having virus symptoms.

- **Fruit number and damage**

Plants from the first field season, 2019, did not produce any fruits. Weather conditions were particularly hot and dry (Tutiempo 2021), and even though plants were watered every two days, they started to die before producing fruits. Thus, fruit number and damage was evaluated only for plants of the second field season, 2020. We recorded the total number of fruits collected at the end of the season for each variety and wild accession. The leading causes of fruit damage were insects (some weevils and Lepidoptera larvae) and rotten fruits. Although it was not the goal of our study, we also recorded the number of fruits removed by birds. Their typical damage was recognized by detaching only the fruits and keeping the peduncle and the bracts intact (Figure S4) (Levey et al. 2006). We did not find any fruits on the soil that had fallen or aborted by the parental plant. Fruits removed by birds were counted once a week from 7 am to 11 am. In the common garden, birds were observed attacking ripe chili fruits (Alfredo Rojas, personal communication). Local residents confirmed that a bird known in the area as "Chiguiro" (Tropical kingbird, family Tyrannidae) is responsible for consuming wild chilies in their gardens and described their typical damage as the one observed during our sampling in the 2020 season.

3.2.4. Field sampling of wild chili accessions

To evaluate the resistance of chiltepin accessions in their natural habitat to herbivore damage and symptoms of virus infesction as well as characterize their fruits according to the capsaicinoids levels, we sampled wild chilies in nine different locations around Puerto Escondido, Oaxaca (Figure S5). The number of sampled plants per location was based on their availability and presence of mature fruits. The locations were: San Pedro (N= 9), La Reforma (N= 5), Los Limones (N= 2), Puerto Escondido (N= 5), San Juan (N= 5), Regadio (N= 4), Manialtepec (N= 6), El Tomatal (N= 8), and Chila (N= 6). At least ten mature fruits per plant were collected. They were used to check for insect damage and to quantify capsaicinoid content.

3.2.5. Quantification of capsaicinoids in chili fruits

To verify the pungency level of the fruits from the wild accessions and from the 2020 common garden experiment, we quantified the capsaicinoids content in chili fruits. Whole fruits from each plant were oven-dried for 48 hours at 60 °C, following the method developed by Collins et al. (1995). Once dried, they were ground with a mortar to obtain a fine powder, and 10 mg was extracted with 1ml of methanol. The mixture was then centrifuged for 5 min at 14 000 rpm; 700 ul of supernatant was collected and further diluted 100'000-fold before HPLC analysis. For each accession, we had five fruits from each plant (1 replicate = 1 plant). We used all sampled plants from the nine locations and eight plants from each treatment for the 2020 common garden experiment. The capsaicin and dihydrocapsaicin contents were analyzed using an Acquity ultra-high-pressure liquid chromatography (UHPLC) system coupled to a Synapt G2 QTOF mass spectrometer (Waters, Milford, MA, USA) controlled by Masslynx 4.1, as described by (Chabaane et al. 2021).

3.2.6. Statistical analysis

All statistical analyses were performed with R statistical software (version 3.5.3; R Development Core Team, 2020), using ANOVA, followed by residual analysis to verify the suitability of distributions of the tested models. Generalized Linear Models (GLM) with a Gaussian distribution followed by post-hoc analysis (Tukey) were used to verify the trichome density, phenolic content in leaves, and capsaicinoid content in fruits. Whiteflies and virus infestations were analyzed using Generalized Linear Mixed Models (GLMMs) under Binomial distribution. To follow the plant growth of wild and domesticated chilies, we used Generalized Linear Mixed Models (GLMMs) with a Gaussian distribution. For repeated measurements, models included plant domestication status (domesticated or wild), plant accession/varieties nested within plants domestication status (to account for natural variation among the three cultivated varieties and the three wild populations), and week as fixed factors. Plots, subplots, and the ID of the plant were included as random factors to account for the repeated measurement structure. The overdispersion of the data was verified, and when necessary, the correction quasibinomial was applied. The sample size and number of replicates for all experiments is indicated directly in the figure captions.

3.3. Results

3.3.1. Effect of chili domestication on trichome density and phenolic content in leaves

- **Trichome density**

Leaves of wild accessions have considerably more trichomes than leaves of domesticated varieties, on both sides of the leaf and at the edge (Fig. 1, upper leaf surface: $F_{[1,70]} = 75.296$, d.f = 1, $P < 0.001$; lower leaf surface: $F_{[1,70]} = 39.302$, d.f = 1, $P < 0.001$; leaf edge: $F_{[1,70]} = 33.296$, d.f = 1, $P < 0.001$). Overall, domesticated plants had three times more trichomes on the leaf edge than on the upper and lower sides. However, trichomes on wild plants are equally distributed throughout the leaf.

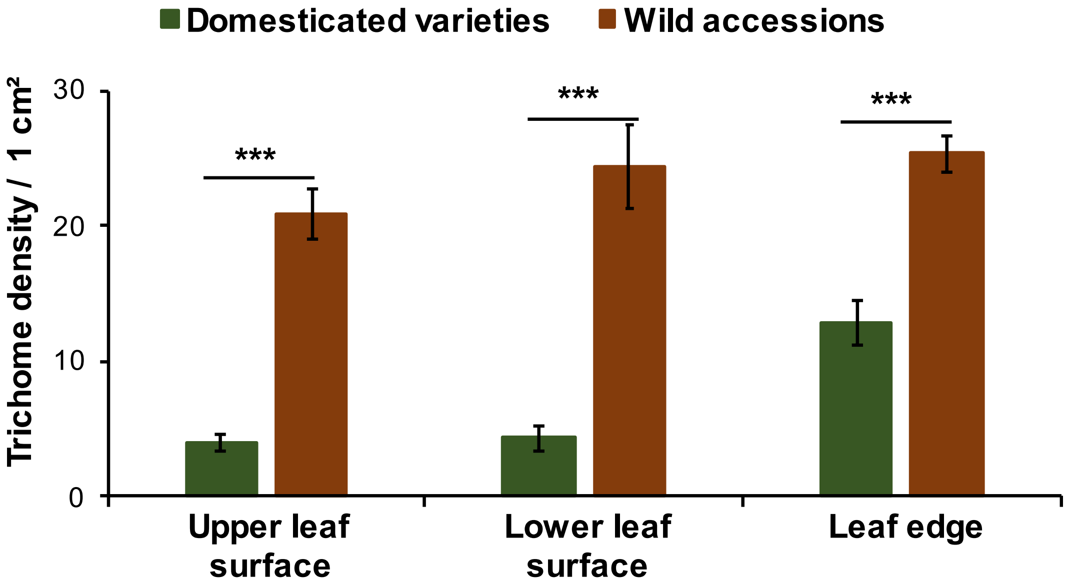


Fig. 1 Trichome density on chili leaves of domesticated varieties (Dulce, Patagonia, and Habanero) and wild accessions (Batopilas, Phoenix, and Sonoran). Stars indicate the difference between treatments (F-test: ***P<0.001). The data from the three domesticated varieties and three wild accessions are pooled for each domestication status, respectively. Sample size: N = 36 leaves/treatment, 12 leaves per variety/wild accession.

- **Phenolic content in leaves**

Phenolic analysis of leaves revealed great quantitative differences between domesticated varieties and wild accessions, both in flavonoids and phenol esters (Fig. 2, flavonoids: $F_{[1,568]} = 7.379$, d.f = 1, $P = 0.006$ and phenol esters: $F_{[1, 169]} = 26.378$, d.f = 1, $P < 0.001$). Leaves of domesticated varieties had 45 % to 50 % more flavonoids and phenol esters than leaves of wild accessions.

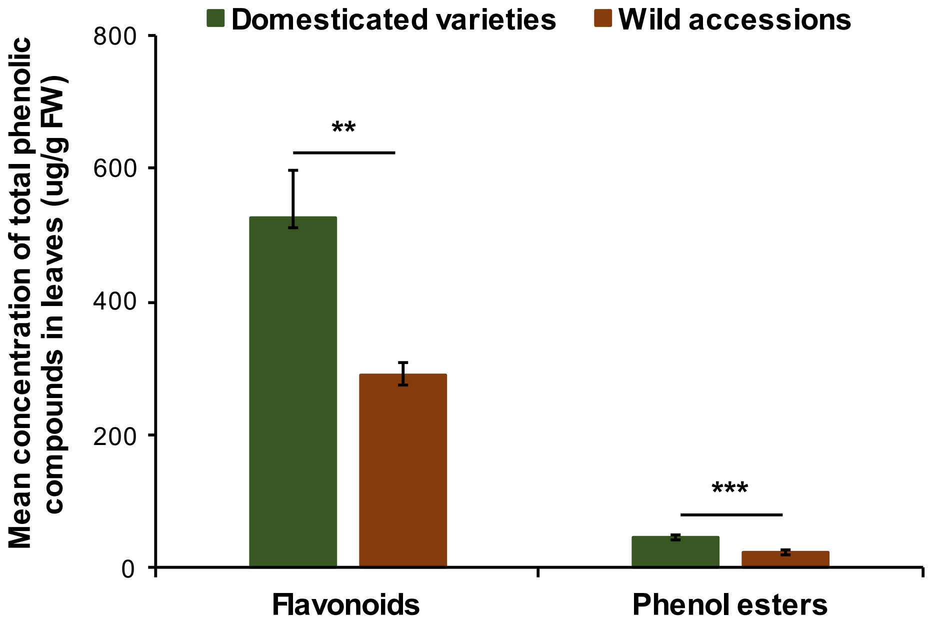


Fig. 2 Total phenolic content in chili leaves of domesticated varieties (Dulce, Patagonia, and Habanero) and wild accessions (Batopilas, Phoenix, and Sonoran). Stars indicate difference between treatments (F-test: **P<0.01, ***P<0.001). The data from the three domesticated varieties and three wild accessions are pooled for each domestication status, respectively. Sample size: N= 30 plants/treatment, 10 plants per variety/wild accession.

3.3.2. Insect damage on wild and domesticated plants in a common garden field experiment

- **Plant growth: number of leaves**

Overall, domesticated plants produced more leaves than wild accessions. This difference in was significant for the following weeks (Fig. 3A, 6th week: $F_{[1,192]} = 45.967$, d.f = 1, P <0.001 and 7th week: $F_{[1, 190]} = 31.109$, d.f = 1, P <0.001 and Fig. 3B, 4th week: $F_{[1, 133]} = 12.146$, d.f = 1, P <0.001; 5th week: $F_{[1, 133]} = 25.526$, d.f = 1, P <0.001 and 6th week: $F_{[1, 130]} = 38.148$, d.f = 1, P <0.001). For 2019 experiment, wild plants had initially more leaves than domesticated plants. This difference is due to the wild accession Bacadehuachi, characterized by many, small sized leaves.

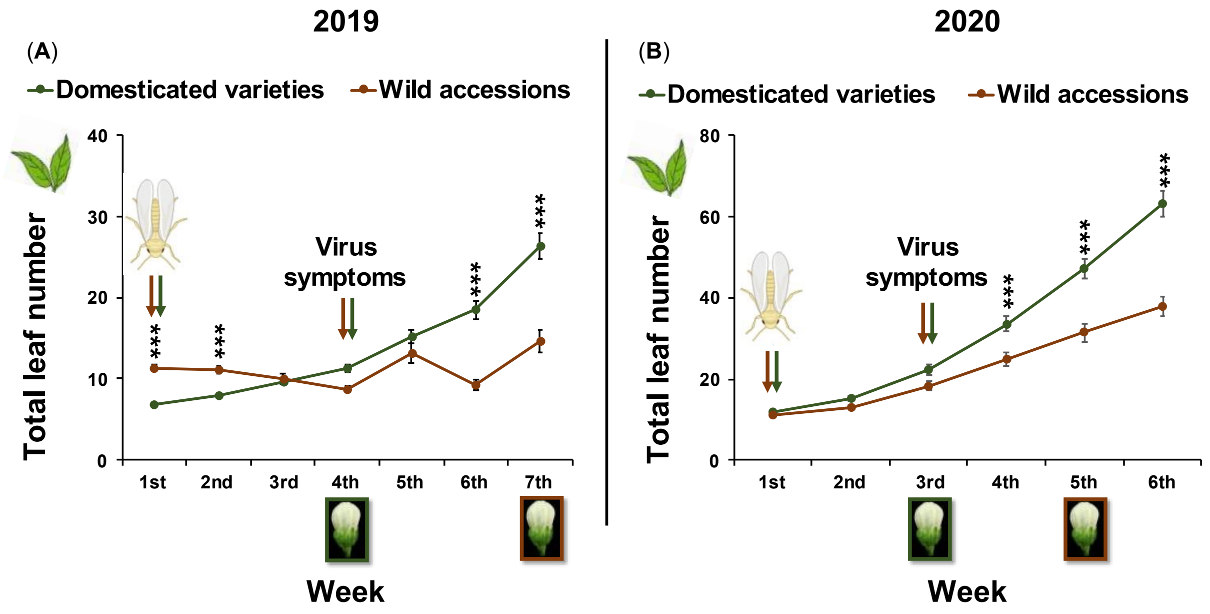


Fig. 3 Plant growth showing total leaf number of domesticated varieties and wild accessions during two field seasons (**A**) 2019 experiment and (**B**) 2020 experiment. Stars indicate the difference between treatments (F-test ***P<0.001). Sample size: Fig 3A. N= 144 plants and Fig 3B. N= 72 plants for each treatment.

- **Leaf damage by chewing insects**

In both field seasons, damage by chewing insects was higher on leaves of domesticated plants than on wild accessions (Fig. 4A, 2019 experiment: $F_{[1,\ 1405]} = 221.005$, d.f = 1, P <0.001 and Fig. 4B, 2020 experiment: $F_{[1,\ 824]} = 104.840$, d.f = 1, P <0.001). In the 2019 experiment, about 17 % for domesticated and 10 % for wild plants of the total leaf was removed. However, damage was minimal in 2020, less than 3% for both plant treatments (Figure 4A).

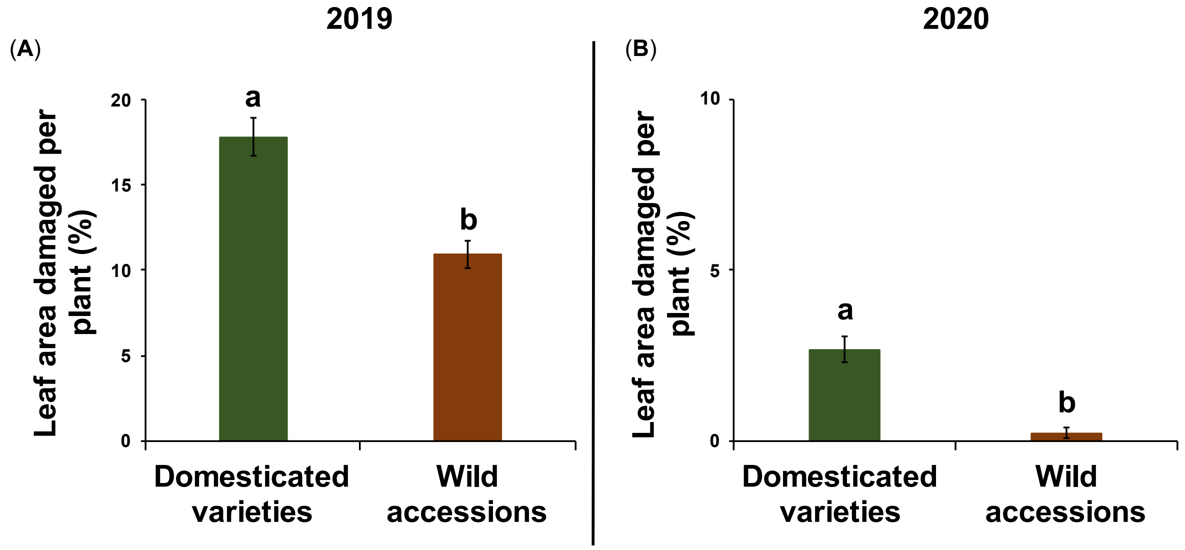


Fig. 4 Leaf area damaged by chewing insects on leaves of domesticated varieties and wild accessions during two field-seasons (**A**) 2019 experiment and (**B**) 2020 experiment. Difference

between treatments is indicated by different letters (F-test ***$P<0.001$). Sample size: Fig 4A. N= 144 plants and Fig 4B. N= 72 plants for each treatment.

- **Whitefly infestation and virus infection on leaves**

Whiteflies were the main herbivore recorded in our common garden. Indeed, at least 60% of domesticated and wild plants were infested during both seasons (Figures 5A and 5B). Overall, whiteflies were more present on wild accessions than on domesticated varieties. This difference was more evident towards the end of the season for the 2019 experiment (Fig 5A. 5th week: $\chi^2 = 244.50$, d.f = 1, P = 0.003, 6th week: $\chi^2 = 261.07$, d.f = 1, P <0.001 and 7th week: $\chi^2 = 257.55$, d.f = 1, P <0.001). However, the same effect was recorded at the beginning of the season for the 2020 experiment (Fig 5B. 1st week: $\chi^2 = 188.63$, d.f = 1, P = 0.002, 2nd week: $\chi^2 = 193.69$, d.f = 1, P = 0.019 and 4th week $\chi^2 = 184.39$, d.f = 1, P = 0.001).

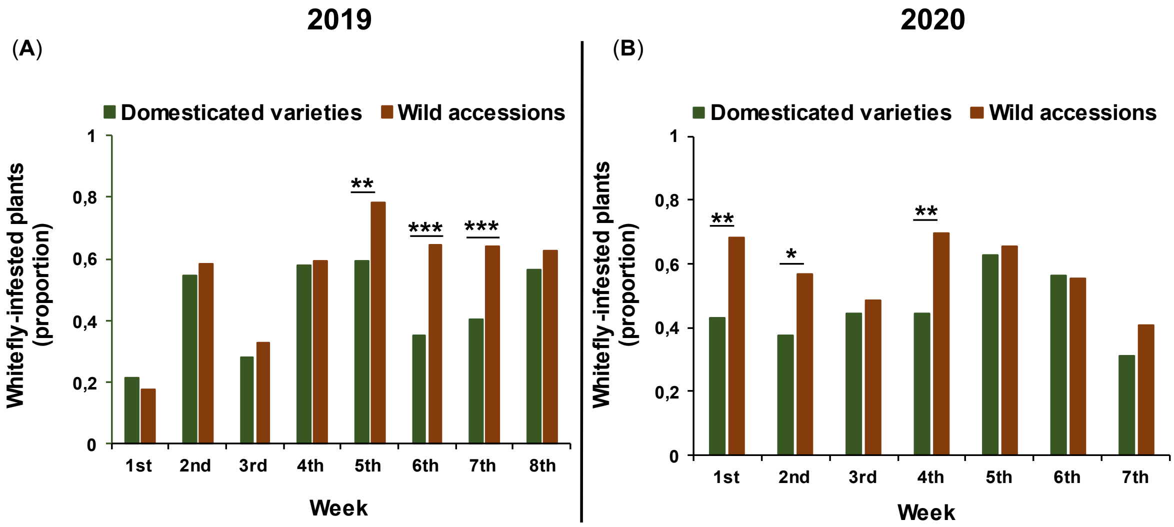


Fig. 5 Proportion of whitefly-infested plants for domesticated varieties and wild accessions per week during two field-seasons (**A**) 2019 experiment and (**B**) 2020 experiment. The proportion of infested plants = Number of plants on which we found whiteflies/total number of plants. Stars indicate differences between treatments within the same week (Chi-test: * P<0.05, **P<0.01, ***P<0.001). Sample size: Fig 5A. N= 144 plants and Fig 5B. N= 72 plants for each treatment.

Virus symptoms appeared on chili plants two to three weeks after whitefly infestation was first recorded, during the 2019 and 2020 experiments (Figures 6. A and B). Interestingly, these results followed an opposite pattern to the one found for whiteflies. Virus infection was significantly higher on domesticated varieties than on wild plants, particularly during the 2019 experiment (Fig 6a. 4th week: $\chi^2 = 159.34$, d.f = 1, P <0.001, 5th week: $\chi^2 = 222.43$, d.f = 1, P <0.001, 6th week: $\chi^2 = 240.40$, d.f = 1, P <0.001 and 7th week $\chi^2 = 241.36$, d.f = 1, P <0.001). During the 2020 experiment, the same effect was found, but the difference between domesticated and wild plants was only significant at the initial period of virus infection, which corresponded to week 3 of sampling (Fig 6b. 3rd week: $\chi^2 = 82.965$, d.f = 1, P = 0.036).

Regarding the level of virus infection, domesticated plants were significantly highly infected compared to wild accessions during the 2019 experiment (Fig. 7A: $\chi^2 = 204.17$, d.f =

1, P <0.001). However, no significant difference among treatments was detected for the second season. Overall, we found that the proportion of highly infected plants was lower for the 2020 experiment than for the first season. This difference is probably due to the lower whitefly-infested plants recorded at the end of the season (Figure 5 B, week 7).

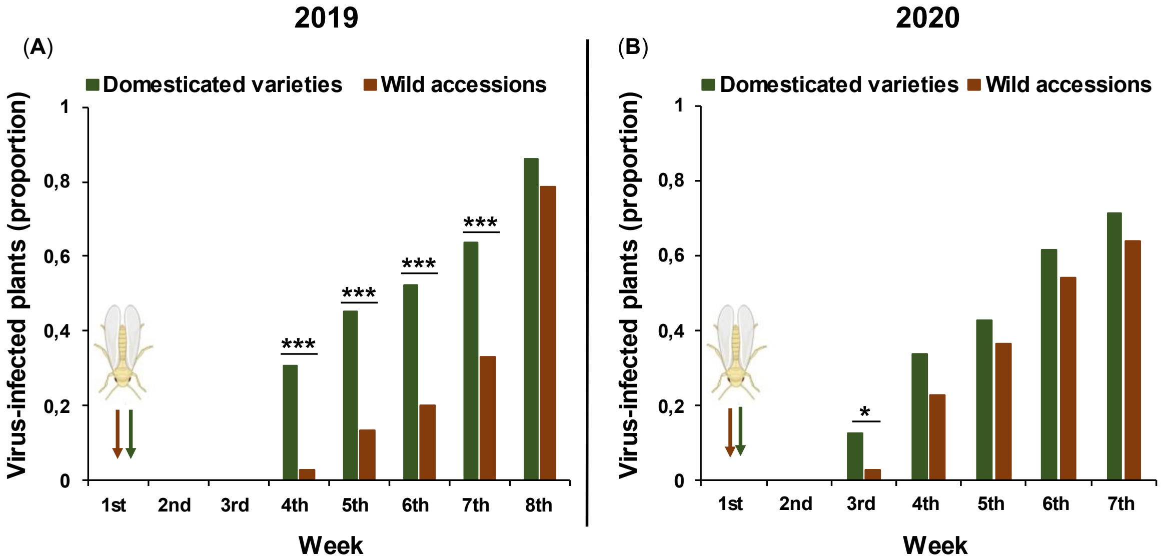


Fig. 6 Proportion of virus-infected plants for domesticated varieties and wild accessions per week during two field-seasons (**A**) 2019 experiment and (**B**) 2020 experiment. The proportion of virus-infected plants = Number of plants with virus symptoms / total number of plants. Stars indicate differences between treatments within the same week (Chi-test: * P<0.05, ***P<0.001). Sample size: Fig 6A. N = 144 plants and Fig 6B. N = 72 plants for each treatment.

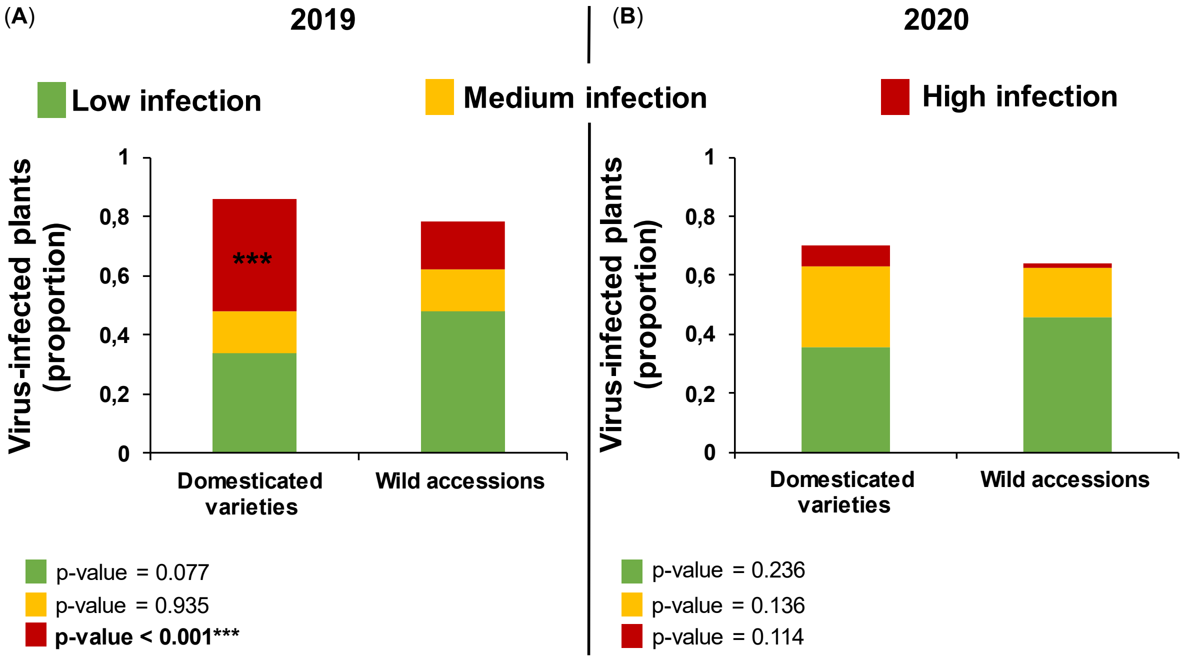


Fig. 7 Virus infection levels on domesticated varieties and wild accessions recorded and the last week of both field-seasons (**A**) 2019 experiment and (**B**) 2020 experiment. Low infection= less than 25 % of total leaves having virus symptoms. Medium infection = between 25 % and 75 % of total leaves having virus symptoms. High infection = more than 75 % of total leaves having virus symptoms. Stars indicate differences between treatments within the same infection level (Chi-test: ***P<0.001). Sample size: Fig 7A. N = 144 plants and Fig 7B. N = 72 plants for each treatment.

- **Fruit number, damage, and capsaicinoids content**

At the end of the 2020 experiment, all domesticated varieties and wild accessions had produced fruits except for the Sonoran wild accession. There were very few insect attacks, only on Dulce and Patagonia varieties: 8.33 % and 0.19 % respectively, of the total number of fruits collected and rotten fruits were recorded exclusively on domesticated varieties. However, it was not possible to tell if the rot was a secondary consequence of insect damage. Moreover, it is interesting to note that despite the highest number of fruits collected of the Patagonia variety and their upright position on the plant, birds attacked only wild fruits from Batopilas and Phoenix accessions (Table 1).

Table 1. Damage on fruits of chili varieties and wild accessions during the 2020 experiment caused by insect herbivores, fungi, and birds (expressed in % of fruits damaged from the total number of fruits collected for the whole season). Photo of the tropical kingbird © Daniel Irons, available at https://ebird.org/species/trokin (accessed on 10 July 2021).

Domestication status	Name	Total number of fruits	Fruits damaged by insects (%)	Rotten fruits (%)	Fruits damaged by birds (%)
Domesticated varieties	Dulce	72	8.33	16.66	-
	Patagonia	1046	0.19	0.09	-
	Habanero	207	-	0.48	-
Wild accessions	Batopilas	166	-	-	4.21
	Phoenix	147	-	-	5.44
	Sonoran	-	-	-	-

The pungency level in fruits revealed a considerable variation in capsaicinoid content across domesticated varieties and wild accessions used for the 2020 common garden experiment (capsaicin: $F_{[4,35]} = 18.27$, d.f = 4, P <0.001; dihydrocapsaicin: $F_{[4,35]} = 21.27$, d.f = 4, P <0.001). As expected, wild accessions had an intermediate level of capsaicinoids between the highly pungent Habanero variety and the mild Patagonia variety. In addition, Habanero fruits had eight times more capsaicin than the Patagonia fruits, whereas no dihydrocapsaicin was detected for this mild variety. The Dulce variety had no capsaicinoids at all (Figure 8).

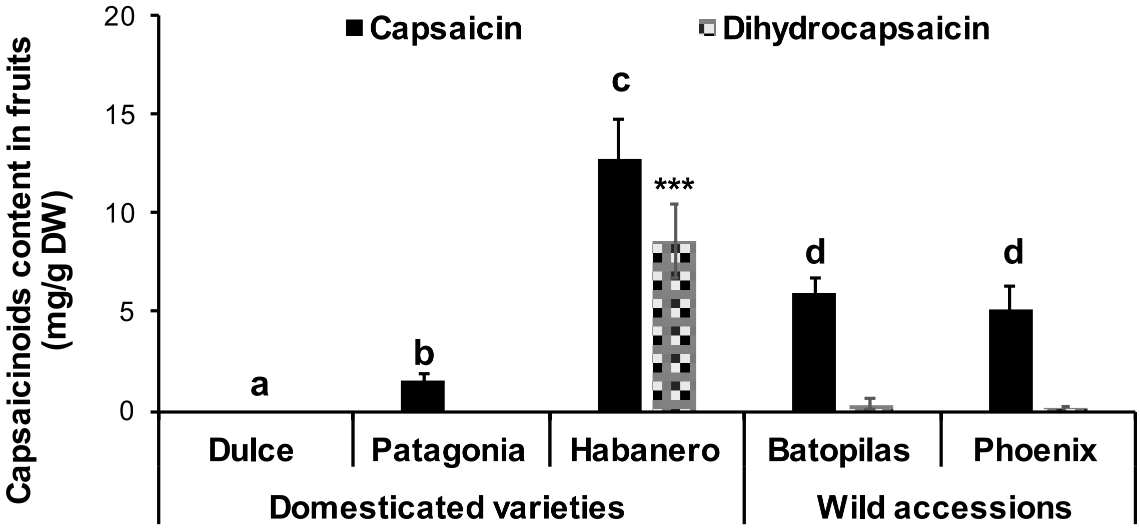


Fig. 8 Mean (±SEM) capsaicinoids content in chili varieties (Dulce, Patagonia, and Habanero) and wild chili accessions (Batopilas and Phoenix) from the 2020 common garden experiment. Significant differences among treatments are indicated by different letters for capsaicin and stars for the dihydrocapsaicin concentrations (F-test, Tukey post-hoc test with Bonferroni correction: P<0.001). The sample size is N= 8 plants and five fruits from each plant were used to quantify capsaicinoids, a total of 40 fruits per treatment.

3.3.3. Field sampling of wild chili accessions

Sampled wild accessions from nine different locations revealed no variation for the dihydrocapsaicin content in chili fruits (Fig 9, $F_{[8,41]} = 1.335$, d.f = 8, P = 0.253). However, fruits from the San Juan location had 40 to 60 % more capsaicin than fruits from the other locations (Fig 9, $F_{[8,41]} = 5.539$, d.f = 8, $P < 0.001$). No significant difference was detected for the capsicin level in fruits among the other locations.

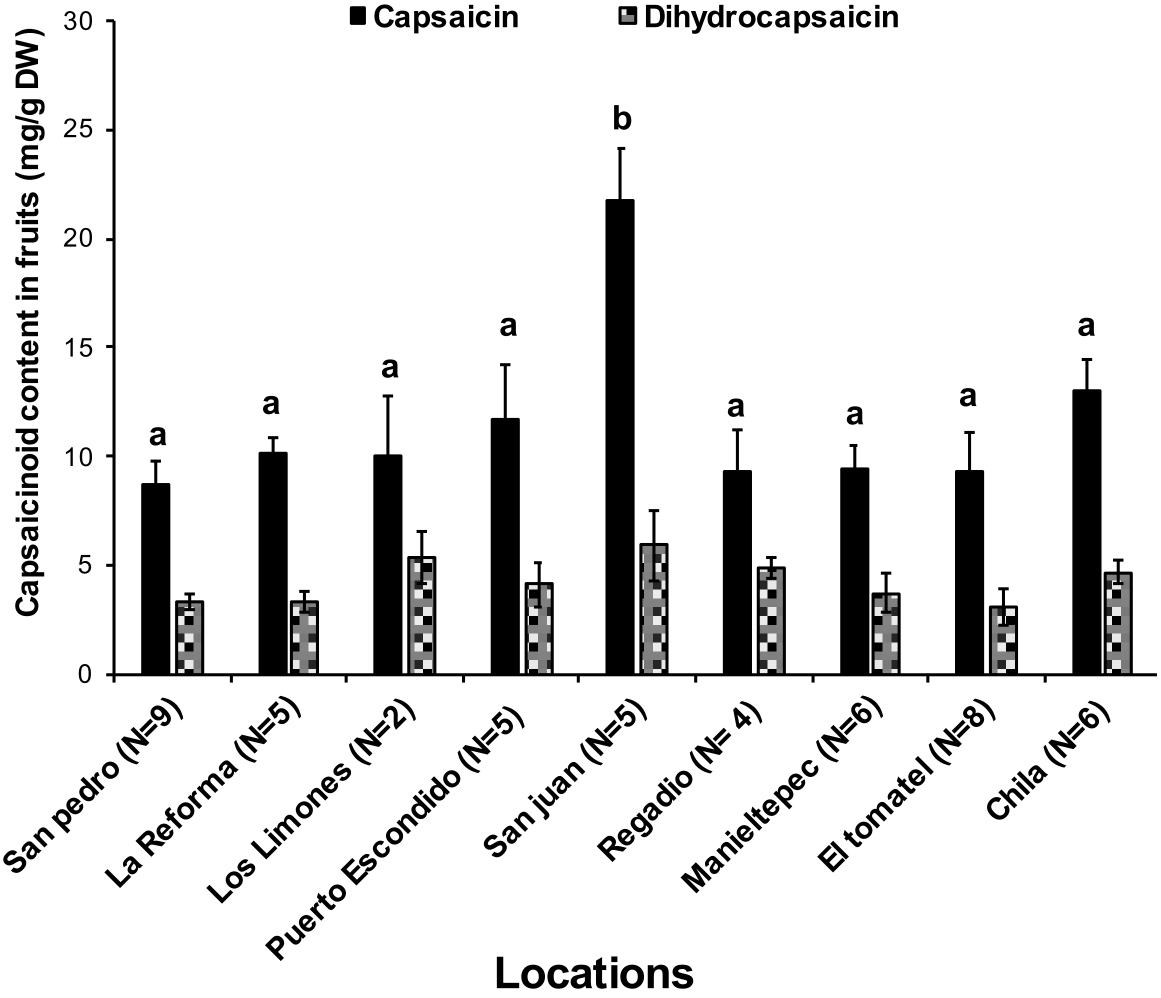


Fig. 9 Mean (±SEM) capsaicinoid content in the sampled wild chili accessions from nine different locations around Puerto Escondido (Oaxaca, Mexico). Significant difference among locations are indicated by different letters for capsaicin (F-test, Tukey post-hoc test with Bonferroni correction: $P<0.001$), and no significant difference among the locations was detected for the dihydrocapsaicin concentrations. The sample size is indicated directly in the figure for each location (N= number of sampled plants and five fruits from each plant were used to quantify capsaicinoids).

3.4. Discussion

Our results reveal that whitefly and the damage caused by their transmitted virus represent chili pepper's primary pest and disease in this natural habitat. Over half of the plants in the common garden were attacked by whiteflies and showed virus symptoms in both seasons 2019 and 2020. Conversely, leaf damage by chewing insects was less than 20% for all plants for the first season and minimal (< 10%) for the second season. Other studies have documented the importance of whitefly infestations in chili plantations worldwide (Firdaus et al. 2011; Nasruddin and Stocks 2014; Torres-Pacheco et al. 1996). In Mexico, the Middle East-Asian B biotype was introduced early in the 1990s, then the Mediterranean Q biotype in 2005, both in Sonora and on the western coast, most probably through Arizona (USA) (Martinez-Carrillo and Brown 2007; McKenzie et al. 2012). The lack of natural enemies in the introduced regions has likely facilitated their success as a problematic pest of this and other vegetable crops, not only in Mexico but in many areas of the world where this pest was introduced (Bellows et al. 1992; Kanakala and Ghanim 2019; Rao et al. 2018). In contrast, chewing herbivores recorded in the common garden such as *Spodoptera latifascia* and *Manduca* spp are originally from Mesoamerica (Kawahara et al. 2013; King and Saunders 1984; Zagatti et al. 1995) and are attacked by a suit of natural enemies that share the same area of origin (Traine et al. 2020). Indeed, previous studies have shown that *S. latifascia* is commonly attacked by hymenopteran parasitoids in this same field site (Bustos-Segura et al. 2020; Cuny et al. 2018).

Interestingly, whitefly infestation was higher on wild accessions than on domesticated varieties (22 % higher in 2019 and 19 % higher in 2020). Yet, considerably fewer wild plants showed symptoms of viral infection (figure 6), revealing some degree of resistance. A plausible explanation for these results could be that morphological or biochemical traits of wild plants' might be attractive for whiteflies but not suitable for transmitting the virus. For example, wild accessions might emit more or different volatiles than domesticated plants resulting in a higher attraction. Various studies have examined the volatile emission from domesticated chili fruits, showing a diverse volatile blend according to the species and fruit maturity (Bogusz Junior et al. 2012; Chitwood et al. 1983; Kollmannsberger et al. 2011; Pino et al. 2011). In another study, Patel et al. (2016) characterized the volatile profiles of ripening fresh fruits from 50 different Peruvian wild accessions and found that the predominant volatiles were terpenes, esters, and hydrocarbons. However, as the corresponding comparison with fruits from domesticated varieties was not done, we do not know the differences, if any, in the volatile profiles between these wild accessions and their domesticated counterparts. To date, no studies have examined the volatile profiles of leaves of wild and cultivated chili plants. However, it was demonstrated that leaves of domesticated varieties and wild plants from other crops emit different volatile signals (de Lange et al. 2016; Gouinguené et al. 2001; Rowen and Kaplan 2016). In our study, whitefly infestation started at the beginning of both seasons before the fruiting stage. Therefore, if volatiles play a role in host location and acceptance by whiteflies, only the leaf volatiles would affect this interaction. This idea remains to be tested.

Additionally, our results showed that whiteflies transmitted viruses have a long latent period since symptoms on plants appeared three weeks after whitefly infestation. Similar latent periods were reported for the Cucurbit Yellow Stunting Disorder virus (CYSDV), also transmitted by whiteflies and infect all major cucurbit crops (Adkins et al. 2009). The lower virus infection rate on wild plants may be due to a higher degree of resistance. It was reported that some plant toxins are involved in the mortality of whiteflies, therefore the resistance against begomoviruses (Zaidi et al. 2017). For example, Shukla et al. (2016) identified a protein, Tma12, from an edible fern *Tectaria macrodonta* (Fee) C. Chr, that was toxic to whiteflies. They also showed that transgenic cotton lines that express this protein were resistant to whitefly infestation in the field and protected plants from the cotton leaf curl virus. In addition, the transmission for some viruses requires that their vectors feed for a long time on the phloem sap (Gray et al. 2014). Therefore, wild accessions might have physical and chemical defensive barriers that prevent the feeding of whiteflies and, consequently, viruses' transmission.

The domestication of chili focused mainly on changes that targeted the fruit (e.g., size, capsaicin level, position, and the deciduous characteristic of the fruit) (Pickersgill 2016; Tewksbury and Nabhan 2001; Tewksbury et al. 2008). However, altered traits in leaves are poorly investigated. Therefore, in the current study, we focused on physical (trichome) and chemical (phenolic compounds) defenses in chili leaves. Our results showed that domestication has also altered these two traits but in the opposite direction. Domestication decreased trichome density, which may explain increased damage by chewing insects on domesticated plants. In another study, Andrade et al. (2017) showed that glandular trichomes in the parental wild accession (LA1401, *Solanum galapagense*) reduced whitefly population compared to the susceptible parent TOM-684 and the F1 population. In our study, both wild accessions and domesticated varieties had only non-glandular trichomes. This may explain why the high trichome density on wild accessions presented a barrier for chewing insects but not for whiteflies. This idea remains to be tested.

Conversely to the pattern found for the trichomes, the content of phenolic compounds was higher on domesticated plants than on wild accessions. This result was contrary to our expectation, as phenolic compounds are commonly associated with plant defense (Chacón-Fuentes et al. 2015; Nicholson and Hammerschmidt 1992; Simmonds 2001). However, phenolic compounds are not always responsible for plant resistance against insect pests. For example, for apple, although domestication has decreased the quantity and diversity of phenolic compounds in fruits, it is not correlated with the performance of the codling moth (*Cydia pomonella*) (Whitehead and Poveda 2019). This finding could be explained by the fact that phenolics, like other secondary metabolites, have more than one function in the plant (Cheynier 2012; Laura et al. 2019). They are involved in the synthesis of fruit color (Boudet 2007), and flavonoids help to mitigate the stress caused by UV radiation by reducing their penetration or acting as quenchers of reactive oxygen species (ROS) (Del Valle et al. 2020). Thus, it is possible that domesticated chilies might need more phenolic compounds in their leaves to protect them against UV stress than wild chilies that naturally grow in the forest or shaded areas (Kraft et al. 2013).

On fruits, the incidence of insects and the secondary attacks caused by fungi were very minimal (< 10 % of total collected fruits) and exclusively on domesticated varieties. It is probably because the fruiting stage coincides with the beginning of the dry season around March, April when herbivore abundance in this site is very low (Bustos-Segura et al. 2020). Likewise, sampled wild chili fruits from nine different locations did not had any insect damage. Interestingly, and although it was not the goal of our study, residents reported that the fruits of the wild chiltepin are intensively attacked by a bird called the locals "*Chiguiro*" a tropical kingbird (family Tyrannidae). In the common garden, we also found that birds attacked only fruits produced by wild plants. This result confirms that chili domestication favored the loss of fruit dispersion by birds by changing their position and removing their deciduous characteristic (Pickersgill 2016). Indeed, fruits of domesticated varieties used in this study remain attached to the plant when ripen. However, wild fruits are deciduous at this stage. Therefore, birds did not remove fruits of Patagonia variety despite their upright position, similar to wild accessions. These findings illustrate very well the consequences of chili domestication on bird fruit selection. This type of study is particularly interesting in regions like Mexico, where chili fields are cultivated adjacent to backyard gardens with wild chili plants. Further work should be done in situ to investigate the vital role of local birds in the ecological restoration of degraded areas due to intensified monoculture farming. Protecting these birds is crucial to preserve chili diversity and ecosystem services for humans (Egerer et al. 2018).

Another potential contribution from the current study is the use of mixed varieties that has been shown to reduce the impact of pests and diseases by increasing the biodiversity in agroecosystems (Barot et al. 2017; Finckh et al. 2000; Ratnadass et al. 2012). Wild plants grow naturally in diversified plant communities, and they are generally not visible to insects (Feeny 1976). However, agriculture favoured the cultivation of a particular plant species or variety in high densities in addition to weeding. These cultural practices contribute to higher plant visibility to herbivores. Therefore, mixing crop varieties that differ in their resistance to insects and diseases might dilute the cues emitted by a single host plant (Barbosa et al. 2009; Barot et al. 2017; Finckh et al. 2000). In our study, the experimental design of the common garden mixed chili varieties with wild accessions that differ in their resistance to insects and viruses. We noticed that this practice had reduced the impact of whiteflies and viruses on our plants compared to the high incidence reported during the survey where only domesticated varieties were planted. The particularity of chili pepper is that wild chili is also widely used by consumers and contributes to locals' income. Therefore, future work should study the diluted effect of using wild chili pepper with mixed chili varieties on pest infestation and virus infection, compared to the monovarietal system.

In conclusion, our study revealed that chili domestication enhanced plant susceptibility to leaf chewing herbivores and the virus transmitted by whiteflies, but surprisingly not to whiteflies. We also found partial support for our hypothesis that predicted that wild accessions are better defended than domesticated varieties. This hypothesis was confirmed only for trichome density but not for phenolic content in leaves, where we found the opposite pattern. Therefore, the consequences of chili domestication on plant-mediated interactions with multiple herbivore species and plant defensive traits are not straightforward. Although it was not the goal of our study, the effects of chili domestication on fruit selection by birds were also reported. Studying these interactions in one of the main domestication centres of chili pepper, where domesticated varieties still coexist with their wild ancestors, can shed light into the role of natural and human-mediated selection in shaping the present-day plant phenotypes (Chen et al. 2017). Further work both in situ and in the laboratory is needed to better understand these complex interactions and critical changes resulting from plant domestication. From an applied perspective, these studies are crucial to develop sustainable pest control strategies for this important crop.

Acknowledgments

We are thankful to the Universidad del Mar of Puerto Escondido (Oaxaca, Mexico) for logistic support during fieldwork. We also thank Alfredo Rojas, Victor Lopez, Carlos Bustos-Segura, Lucas Malacari, Charlyne Jaccard, Nicolas Marguier, Anthony Pignal, and Lauren Maynard for their help in the field and sampling wild chili accessions and Gaëtan Glauser for his assistance with the chemical analysis. We are also very grateful to residents and farmers of Puerto Escondido and the neighbouring villages for their kindness and willingness to share their knowledge and stories about chili. The authors declare no conflict of interest. This research was financed by a grant from the Swiss National Science Foundation (Project No: 310030_197463) awarded to Betty Benrey, a grant from the University of Neuchatel "Egalité des chances" and a Swiss Government Excellence Scholarship for foreign students awarded to Yosra Chabaane.

References

Abdala-Roberts L, Berny-Mier y Terán JC, Moreira X, Durán-Yáñez A, Tut-Pech F (2015) Effects of pepper (*Capsicum chinense*) genotypic diversity on insect herbivores. Agricultural and Forest Entomology 17:433-438. doi:https://doi.org/10.1111/afe.12125

Adkins S, Webster CG, Baker CA, Weaver R, Rosskopf EN, Turechek WW (2009) Detection of three whitefly-transmitted viruses infecting the cucurbit weed *Cucumis melo* var. *dudaim* in Florida Plant Health Progress 10:39 doi:https://doi.org/10.1094/PHP-2009-1118-01-BR

Andrade MC, da Silva AA, Neiva IP, Oliveira IRC, De Castro EM, Francis DM, Maluf WR (2017) Inheritance of type IV glandular trichome density and its association with whitefly resistance from *Solanum galapagense* accession LA1401 Euphytica 213:52 doi:https://doi.org/10.1007/s10681-016-1792-1

Aragón-Cuevas F et al. (2004) In situ conservation and participatory breeding of Milpa inOaxaca, Mexico Manejo de la diversidad de los cultivos en los agroecosistemas radicionales:124-130

Ballina-Gomez H, Latournerie-Moreno L, Ruiz-Sanchez E, Perez-Gutierrez A, Rosado-Lugo G (2013) Morphological characterization of *Capsicum annuum* L. accessions from southern Mexico and their response to the Bemisia tabaci-Begomovirus complex Chilean journal of agricultural research 73:329-338 doi:http://dx.doi.org/10.4067/S0718-58392013000400001

Barbosa P, Hines J, Kaplan I, Martinson H, Szczepaniec A, Szendrei Z (2009) Associational resistance and associational susceptibility: having right or wrong neighbors Annual review of ecology, evolution, and systematics 40:1-20 doi:https://doi.org/10.1146/annurev.ecolsys.110308.120242

Barot S et al. (2017) Designing mixtures of varieties for multifunctional agriculture with the help of ecology. A review Agronomy for Sustainable Development 37:13 doi:https://doi.org/10.1007/s13593-017-0418-x

Barro PJD, Liu S-S, Boykin LM, Dinsdale AB (2011) *Bemisia tabaci*: A Statement of Species Status Annual Review of Entomology 56:1-19 doi:10.1146/annurev-ento-112408-085504

Basu A (2019) *Bemisia tabaci* (Gennadius): crop pest and the principal whitefly vector of plant viruses. CRC Press,

Bellotti AC, Arias B (2001) Host plant resistance to whiteflies with emphasis on cassava as a case study Crop Protection 20:813-823 doi:https://doi.org/10.1016/S0261-2194(01)00113-2

Bellows T et al. (1992) Biological control of ash whitefly: a success in progress California Agriculture 46:24-28

Benrey B, Callejas A, Rios L, Oyama K, Denno RF (1998) The Effects of Domestication of Brassica and Phaseolus on the Interaction between Phytophagous Insects and Parasitoids. Biological Control 11:130-140 doi:https://doi.org/10.1006/bcon.1997.0590

Berlinger M (1986) Host plant resistance to Bemisia tabaci Agriculture, Ecosystems & Environment 17:69-82 doi:https://doi.org/10.1016/0167-8809(86)90028-9

Bogusz Junior S, Tavares AM, Filho JT, Zini CA, Godoy HT (2012) Analysis of the volatile compounds of Brazilian chilli peppers (*Capsicum* spp.) at two stages of maturity by solid phase micro-extraction and gas chromatography-mass spectrometry Food Research International 48:98-107 doi:https://doi.org/10.1016/j.foodres.2012.02.005

Bosland PW, Votava EJ, Votava EM (2012) Peppers: vegetable and spice capsicums vol 22. Cabi. doi:10.1080/01140671.2012.745161

Boudet A-M (2007) Evolution and current status of research in phenolic compounds Phytochemistry 68:2722-2735 doi:https://doi.org/10.1016/j.phytochem.2007.06.012

Bustos-Segura C, Cuny MA, Benrey B (2020) Parasitoids of leaf herbivores enhance plant fitness and do not alter caterpillar-induced resistance against seed beetles Functional Ecology 34:586-596 doi:https://doi.org/10.1111/1365-2435.13478

Carrizo García C, Sterpetti M, Volpi P, Ummarino M, Saccardo F (2013) Wild Capsicums: identification and in situ analysis of Brazilian species Breakthroughs in the genetics and breeding of Capsicum and eggplant Edited by S Lanteri, and GL Rotino:205-213

Chabaane Y, Marques Arce C, Glauser G, Benrey B (2021) Altered capsaicin levels in domesticated chili pepper varieties affect the interaction between a generalist herbivore and its ectoparasitoid Journal of Pest Science doi:https://doi.org/10.1007/s10340-021-01399-8

Chacón-Fuentes M, Parra L, Rodriguez-Saona C, Seguel I, Ceballos R, Quiroz A (2015) Domestication in Murtilla (Ugni molinae) Reduced Defensive Flavonol Levels but Increased Resistance Against a Native Herbivorous Insect Environmental Entomology 44:627-637 doi:https://doi.org/10.1093/ee/nvv040

Chen CS, Zhao C, Wu ZY, Liu GF, Yu XP, Zhang PJ (2021) Whitefly-induced tomato volatiles mediate host habitat location of the parasitic wasp Encarsia formosa, and enhance its efficacy as a bio-control agent Pest Management Science 77:749-757 doi:https://doi.org/10.1002/ps.6071

Chen YH, Gols R, Stratton CA, Brevik KA, Benrey B (2015) Complex tritrophic interactions in response to crop domestication: predictions from the wild Entomologia Experimentalis et Applicata 157:40-59

Chen YH, Shapiro LR, Benrey B, Cibrián-Jaramillo A (2017) Back to the origin: in situ studies are needed to understand selection during crop diversification. Frontiers in Ecology and Evolution 5:125. doi:https://doi.org/10.3389/fevo.2017.00125

Chen YH, Welter SC (2002) Abundance of a Native Moth Homoeosoma electellum (Lepidoptera: Pyralidae) and Activity of Indigenous Parasitoids in Native and Agricultural Sunflower Habitats Environmental Entomology 31:626-636 doi:https://doi.org/10.1603/0046-225X-31.4.626

Cheynier V (2012) Phenolic compounds: from plants to foods Phytochemistry Reviews 11:153-177 doi:https://doi.org/10.1007/s11101-012-9242-8

Chitwood RL, Pangborn RM, Jennings W (1983) GC/MS and sensory analysis of volatiles from three cultivars of Capsicum Food Chemistry 11:201-216 doi:https://doi.org/10.1016/0308-8146(83)90103-6

Collins MD, Wasmund LM, Bosland PW (1995) Improved method for quantifying capsaicinoids in *Capsicum* using high-performance liquid chromatography. HortScience 30:137-139. doi:https://doi.org/10.21273/HORTSCI.30.1.137

Cuny MA, Gendry J, Hernández-Cumplido J, Benrey B (2018) Changes in plant growth and seed production in wild lima bean in response to herbivory are attenuated by parasitoids. Oecologia 187:447-457. doi:https://doi.org/10.1007/s00442-018-4119-1

Daryanto A, Hidayat P, Maharijaya A Heterosis of seedling traits and their correlation to aphids infestation in chili pepper. In: IOP Conference Series: Earth and Environmental Science, 2020. vol 1. IOP Publishing, p 012057

Dávila-Flores AM, DeWitt TJ, Bernal JS (2013) Facilitated by nature and agriculture: performance of a specialist herbivore improves with host-plant life history evolution, domestication, and breeding Oecologia 173:1425-1437 doi:https://doi.org/10.1007/s00442-013-2728-2

De Barro PJ, Hidayat SH, Frohlich D, Subandiyah S, Ueda S (2008) A virus and its vector, pepper yellow leaf curl virus and Bemisia tabaci, two new invaders of Indonesia Biological Invasions 10:411-433 doi:https://doi.org/10.1007/s10530-007-9141-x

de Lange ES, Farnier K, Gaudillat B, Turlings TC (2016) Comparing the attraction of two parasitoids to herbivore-induced volatiles of maize and its wild ancestors, the teosintes Chemoecology 26:33-44

Del Valle JC, Buide ML, Whittall JB, Valladares F, Narbona E (2020) UV radiation increases phenolic compound protection but decreases reproduction in Silene littorea PloS one 15:e0231611 doi:https://doi.org/10.1371/journal.pone.0231611

Egerer MH, Fricke EC, Rogers HS (2018) Seed dispersal as an ecosystem service: frugivore loss leads to decline of a socially valued plant, Capsicum frutescens Ecological applications 28:655-667 doi:https://doi.org/10.1002/eap.1667

Elbert A, Nauen R (2000) Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids Pest Management Science: formerly Pesticide Science 56:60-64 doi:https://doi.org/10.1002/(SICI)1526-4998(200001)56:1%3C60::AID-PS88%3E3.0.CO;2-K

Feeny P (1976) Plant Apparency and Chemical Defense. In: Wallace JW, Mansell RL (eds) Biochemical Interaction Between Plants and Insects. Springer US, Boston, MA, pp 1-40. doi:10.1007/978-1-4684-2646-5_1

Fernandez AR, Sáez A, Quintero C, Gleiser G, Aizen MA (2021) Intentional and unintentional selection during plant domestication: herbivore damage, plant defensive traits and nutritional quality of fruit and seed crops New Phytologist doi:https://doi.org/10.1111/nph.17452

Finckh M et al. (2000) Cereal variety and species mixtures in practice, with emphasis on disease resistance Agronomie 20:813-837 doi:10.1051/agro:2000177

Firdaus S, Van Heusden A, Harpenas A, Supena ED, Visser RG, Vosman B (2011) Identification of silverleaf whitefly resistance in pepper Plant Breeding 130:708-714 doi:https://doi.org/10.1111/j.1439-0523.2011.01894.x

Garvey M, Creighton C, Kaplan I (2020) Pepper domestication enhances parasitoid recruitment to herbivore-damaged plants Arthropod-Plant Interactions 14:695-703 doi:https://doi.org/10.1007/s11829-020-09788-z

Gepts P (2004) Crop domestication as a long-term selection experiment. Plant breeding reviews 24:1-44. doi:10.1002/9780470650288

Gols R, Bukovinszky T, Van Dam NM, Dicke M, Bullock JM, Harvey JA (2008) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. Journal of Chemical Ecology 34:132-143. doi:https://doi.org/10.1007/s10886-008-9429-z

Gouinguené S, Degen T, Turlings TC (2001) Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte) Chemoecology 11:9-16 doi:https://doi.org/10.1007/PL00001832

Gray S, Cilia M, Ghanim M (2014) Circulative,“nonpropagative” virus transmission: an orchestra of virus-, insect-, and plant-derived instruments Advances in virus research 89:141-199 doi:https://doi.org/10.1016/B978-0-12-800172-1.00004-5

Hamilton R, Edwardson J, Francki R, Hsu H, Hull R, Koenig R, Milne R (1981) Guidelines for the identification and characterization of plant viruses Journal of General Virology 54:223-241 doi:https://doi.org/10.1099/0022-1317-54-2-223

Hammer K (1984) Das domestikationssyndrom Die Kulturpflanze 32:11-34

Hasanuzzaman ATM, Islam MN, Liu F-H, Cao H-H, Liu T-X (2017) Leaf Chemical Compositions of Different Eggplant Varieties Affect Performance of Bemisia tabaci (Hemiptera: Aleyrodidae) Nymphs and Adults Journal of Economic Entomology 111:445-453 doi:10.1093/jee/tox333

Hasanuzzaman ATM, Islam MN, Zhang Y, Zhang C-Y, Liu T-X (2016) Leaf morphological characters can be a factor for intra-varietal preference of whitefly Bemisia tabaci (Hemiptera: Aleyrodidae) among eggplant varieties PLoS One 11:e0153880 doi:https://doi.org/10.1371/journal.pone.0153880

Idris AB, Grafius E (1996) Effects of Wild and Cultivated Host Plants on Oviposition, Survival, and Development of Diamondback Moth (Lepidoptera: Plutellidae) and Its Parasitoid

Diadegma insulare (Hymenoptera: Ichneumonidae) Environmental Entomology 25:825-833 doi:https://doi.org/10.1093/ee/25.4.825

Kanakala S, Ghanim M (2019) Global genetic diversity and geographical distribution of Bemisia tabaci and its bacterial endosymbionts PLoS One 14:e0213946

Kawahara AY et al. (2013) Evolution of *Manduca sexta* hornworms and relatives: Biogeographical analysis reveals an ancestral diversification in Central America Molecular Phylogenetics and Evolution 68:381-386

King AB, Saunders JL (1984) The invertebrate pests of annual food crops in Central America: A guide to their recognition and control. Bib. Orton IICA/CATIE,

Kollmannsberger H, Rodríguez-Burruezo A, Nitz S, Nuez F (2011) Volatile and capsaicinoid composition of ají (Capsicum baccatum) and rocoto (Capsicum pubescens), two Andean species of chile peppers Journal of the Science of Food and Agriculture 91:1598-1611 doi:https://doi.org/10.1002/jsfa.4354

Kraft KH et al. (2014) Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico Proceedings of the National Academy of Sciences 111:6165-6170

Kraft KH, de Jesús Luna-Ruíz J, Gepts P (2013) A new collection of wild populations of *Capsicum* in Mexico and the southern United States Genetic Resources and Crop Evolution 60:225-232 doi:https://doi.org/10.1007/s10722-012-9827-5

Laura A, Moreno-Escamilla JO, Rodrigo-García J, Alvarez-Parrilla E (2019) Phenolic compounds. In: Postharvest physiology and biochemistry of fruits and vegetables. Elsevier, pp 253-271. doi:https://doi.org/10.1016/B978-0-12-813278-4.00012-9

Levey DJ, Tewksbury JJ, Cipollini ML, Carlo TA (2006) A field test of the directed deterrence hypothesis in two species of wild chili Oecologia 150:61-68 doi:https://doi.org/10.1007/s00442-006-0496-y

Marina Clemente JA, Palacios Torres RE, Martínez Castro CJ, Javier López L, Aguilera Morales ME (2020) Rentabilidad económica de la producción del chile habanero con mezcla de sustratos y fertilización orgánica en invernadero REPOSITORIO NACIONAL CONACYT

Martinez-Carrillo JL, Brown JK (2007) Note: First report of the Q biotype of *Bemisia tabaci* in Southern Sonora, Mexico Phytoparasitica 35:282

Maruthi MN, Rekha AR, Mirza SH, Alam SN, Colvin J (2007) PCR-based detection and partial genome sequencing indicate high genetic diversity in Bangladeshi begomoviruses and their whitefly vector, *Bemisia tabaci* Virus Genes 34:373-385 doi:https://doi.org/10.1007/s11262-006-0027-2

McKenzie CL et al. (2012) Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in North America after the Q invasion Journal of Economic Entomology 105:753-766 doi:https://doi.org/10.1603/EC11337

Meyer RS, DuVal AE, Jensen HR (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. New Phytologist 196:29-48. doi:https://doi.org/10.1111/j.1469-8137.2012.04253.x

Morales FJ, Anderson PK (2001) The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America Archives of Virology 146:415-441 doi:https://doi.org/10.1007/s007050170153

Moreira X, Mooney KA, Rasmann S, Petry WK, Carrillo-Gavilán A, Zas R, Sampedro L (2014) Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences Ecology Letters 17:537-546 doi:https://doi.org/10.1111/ele.12253

Muñoz-Ramírez LS, Peña-Yam LP, Avilés-Viñas SA, Canto-Flick A, Guzmán-Antonio AA, Santana-Buzzy N (2018) Behavior of the hottest chili peppers in the world cultivated in Yucatan, Mexico. HortScience 53:1772-1775. doi:https://doi.org/10.21273/HORTSCI13574-18

Nasruddin A et al. (2020) Effects of Mulch Type, Plant Cultivar, and Insecticide Use on Sweet Potato Whitefly Population in Chili Pepper Scientifica 2020:6428426 doi:https://doi.org/10.1155/2020/6428426

Nasruddin A, Stocks IC (2014) First report of economic injury due to the spiraling whitefly (Hemiptera: Aleyrodidae) on pepper in Indonesia Florida Entomologist 97:1255-1259 doi:https://doi.org/10.1653/024.097.0337

Nicholson RL, Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance Annual review of phytopathology 30:369-389 doi:https://doi.org/10.1146/annurev.py.30.090192.002101

Oriani MAdG, Vendramim JD (2010) Influence of trichomes on attractiveness and ovipositional preference of *Bemisia tabaci* (Genn.) B biotype (Hemiptera: Aleyrodidae) on tomato genotypes Neotropical Entomology 39:1002-1007 doi:https://doi.org/10.1590/S1519-566X2010000600024

Ozores-Hampton M, Boyd NS, McAvoy EJ, Smith HA, Vallad GE, Bell S (2014) Pepper production Vegetable and Small Fruit Production Handbook of Florida Gainesville, FL: IFAS:137-150

Padhi GK, Maity L, Chattopadhyay A, Samanta A (2017) Population dynamics of whitefly (*Bemisia tabaci* Genn.) in chilli and screening of genotypes against chilli leaf curl virus J Entomol Zool Stud 5:104-107

Patel K, Ruiz C, Calderon R, Marcelo M, Rojas R (2016) Characterisation of volatile profiles in 50 native Peruvian chili pepper using solid phase microextraction–gas chromatography mass spectrometry (SPME–GCMS) Food Research International 89:471-475 doi:https://doi.org/10.1016/j.foodres.2016.08.023

Pereira-Dias L, Vilanova S, Fita A, Prohens J, Rodríguez-Burruezo A (2019) Genetic diversity, population structure, and relationships in a collection of pepper (*Capsicum* spp.) landraces from the Spanish centre of diversity revealed by genotyping-by-sequencing (GBS) Horticulture research 6:1-13 doi:https://doi.org/10.1038/s41438-019-0132-8

Perring TM (2001) The *Bemisia tabaci* species complex Crop Protection 20:725-737 doi:https://doi.org/10.1016/S0261-2194(01)00109-0

Pickersgill B (1997) Genetic resources and breeding of *Capsicum* spp Euphytica 96:129-133

Pickersgill B (2016) Chile Peppers (Capsicum spp.). In: Lira R, Casas A, Blancas J (eds) Ethnobotany of Mexico: Interactions of People and Plants in Mesoamerica. Springer New York, New York, NY, pp 417-437. doi:https://doi.org/10.1007/978-1-4614-6669-7_17

Pino J, Fuentes V, Barrios O (2011) Volatile constituents of Cachucha peppers (*Capsicum chinense* Jacq.) grown in Cuba Food Chemistry 125:860-864 doi:https://doi.org/10.1016/j.foodchem.2010.08.073

Rao N, Roshan DR, Rao GK, Ramanandam G (2018) A review on rugose spiralling whitefly, Aleurodicus rugioperculatus martin (Hemiptera: Aleyrodidae) in India Journal of Pharmacognosy and Phytochemistry 7:948-953

Ratnadass A, Fernandes P, Avelino J, Habib R (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review Agronomy for sustainable development 32:273-303 doi:https://doi.org/10.1007/s13593-011-0022-4

Rodriguez-Saona C, Cloonan KR, Sanchez-Pedraza F, Zhou Y, Giusti MM, Benrey B (2019) Differential Susceptibility of Wild and Cultivated Blueberries to an Invasive Frugivorous Pest Journal of Chemical Ecology 45:286-297 doi:https://doi.org/10.1007/s10886-018-1042-1

Rosen R et al. (2015) Persistent, circulative transmission of begomoviruses by whitefly vectors Current Opinion in Virology 15:1-8 doi:https://doi.org/10.1016/j.coviro.2015.06.008

Rowen E, Kaplan I (2016) Eco-evolutionary factors drive induced plant volatiles: a meta-analysis New Phytologist 210:284-294 doi:https://doi.org/10.1111/nph.13804

Scoville WL (1912) Note on capsicums. Journal of the American Pharmaceutical Association 1:453-454. doi:https://doi.org/10.1002/jps.3080010520

Seal DR, Martin CG (2016) Pepper weevil (Coleoptera: Curculionidae) preferences for specific pepper cultivars, plant parts, fruit colors, fruit sizes, and timing. Insects 7:9 doi:https://doi.org/10.3390/insects7010009

Sharma H, Ortiz R (2002) Host plant resistance to insects: an eco-friendly approach for pest management and environment conservation Journal of Environmental Biology 23:111-135

Shukla AK et al. (2016) Expression of an insecticidal fern protein in cotton protects against whitefly Nature Biotechnology 34:1046-1051 doi:https://doi.org/10.1038/nbt.3665

Simmonds MS (2001) Importance of flavonoids in insect–plant interactions: feeding and oviposition Phytochemistry 56:245-252 doi:https://doi.org/10.1016/S0031-9422(00)00453-2

Taggar GK, Gill RS (2016) Host plant resistance in Vigna sp. towards whitefly, *Bemisia tabaci* (Gennadius): a review Entomologia Generalis 36:1-24

Taher D, Ramasamy S, Prohens J, Rakha M (2020) Screening cultivated eggplant and wild relatives for resistance to sweetpotato whitefly (*Bemisia tabaci*) and to two-spotted spider mite (*Tetranychus urticae*) Euphytica 216:157 doi:https://doi.org/10.1007/s10681-020-02692-w

Tewksbury JJ, Nabhan GP (2001) Directed deterrence by capsaicin in chillies. Nature 412:403-404. doi:https://doi.org/10.1038/35086653

Tewksbury JJ, Reagan KM, Machnicki NJ, Carlo TA, Haak DC, Peñaloza ALC, Levey DJ (2008) Evolutionary ecology of pungency in wild chilies. Proceedings of the National Academy of Sciences 105:11808-11811. doi:https://doi.org/10.1073/pnas.0802691105

Torres-Pacheco I, Garzón-Tiznado JA, Brown JK, Becerra-Flora A, Rivera-Bustamante RF (1996) Detection and distribution of geminiviruses in Mexico and the southern United States Phytopathology 86:1186-1192

Traine J, Cuny MA, Bustos-Segura C, Benrey B (2020) The interaction between host and host plant influences the oviposition and performance of a generalist ectoparasitoid. Entomologia Experimentalis et Applicata 169:133-144. doi:https://doi.org/10.1111/eea.12949

Tutiempo (2021) Clima Puerto Escondido, Datos climáticos: 1988 - 2021

Urbaneja-Bernat P, Polk D, Sanchez-Pedraza F, Benrey B, Salamanca J, Rodriguez-Saona C (2020) Non-crop habitats serve as a potential source of spotted-wing drosophila (Diptera: Drosophilidae) to adjacent cultivated highbush blueberries (Ericaceae) The Canadian Entomologist 152:474-489 doi:10.4039/tce.2020.2

Whitehead SR, Poveda K (2019) Resource allocation trade-offs and the loss of chemical defences during apple domestication Annals of Botany 123:1029-1041 doi:https://doi.org/10.1093/aob/mcz010

Whitehead SR, Turcotte MM, Poveda K (2017) Domestication impacts on plant–herbivore interactions: a meta-analysis Phil Trans R Soc B 372:20160034

Zagatti P, Lalanne-Cassou B, le Duchat d'Aubigny J (1995) Catalogue of the Lepidoptera of the French Antilles. INRA

Zaidi SS-e-A, Briddon RW, Mansoor S (2017) Engineering Dual Begomovirus-*Bemisia tabaci* Resistance in Plants Trends in Plant Science 22:6-8 doi:https://doi.org/10.1016/j.tplants.2016.11.005.

Supplementary material

Fig. S1 Wild chilies growing in two backyard gardens in Puerto Escondido.

Table S1. Summary of domesticated varieties and wild accessions used for 2019 and 2020 common garden experiments in Puerto Escondido (Oaxaca, Mexico)

Domestication status	**2019 experiment**		**2020 experiment**	
	Name	Description	Name	Description
Domesticated varieties	Poblano	Non-pungent	Dulce	Non-pungent
	Jalapeno	Mild	Patagonia	Mild
	Habanero	Highly-pungent	Habanero	Highly-pungent
Wild accessions	Batopillas	Chihuahua, Mexico.	Batopillas	-
	Bacadehuachi	Opata homelands, NW Mexico	Pheonix	Northern Sonoran, Mexico
	Sonoran	Central Sonoran, Mexico	Sonoran	-

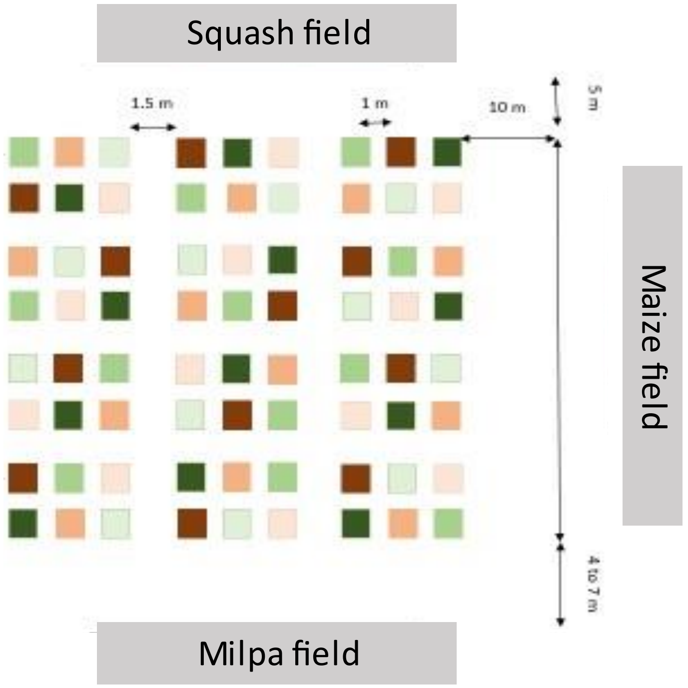


Fig. S2 Experimental design for the chili common garden experiment conducted in Puerto Escondido (Oaxaca, Mexico) for 2019 and 2020 experiments. Green squares represent three different domesticated varieties, and squares in brown refer to three different wild accessions. Each subplot (=1 square) represents three plants for 2019 (N=216 plants) and two plants for 2020 (N=144 plants).

Table S2. Insect diversity attacking wild and domesticated chilies in the common garden

Season	**Chewing insects**	**Sucking insects**	**Natural enemies**
2019 experiment	*Spodoptora latifascia* *Diabrotica* spp, *Acalima* spp	Aphids Mealybug, Leaf miner	Parasitoids Ladybirds, Spiders
2020 experiment	*S. latifascia*, *Manduca* spp *Diabrotica* spp, *Acalima* spp	Leafhoppers Aphids, Leaf miner	Parasitoids (e.g. *Cotesia* spp), Ants Spiders and ladybirds

Fig. S3 Virus symptoms (foliar yellow mosaics and leaf curling) on the cultivated Habanero variety (left) and the wild accession Batopilas (right).

Fig. S4 Typical damage by birds where only the peduncle and bracts remain intact.

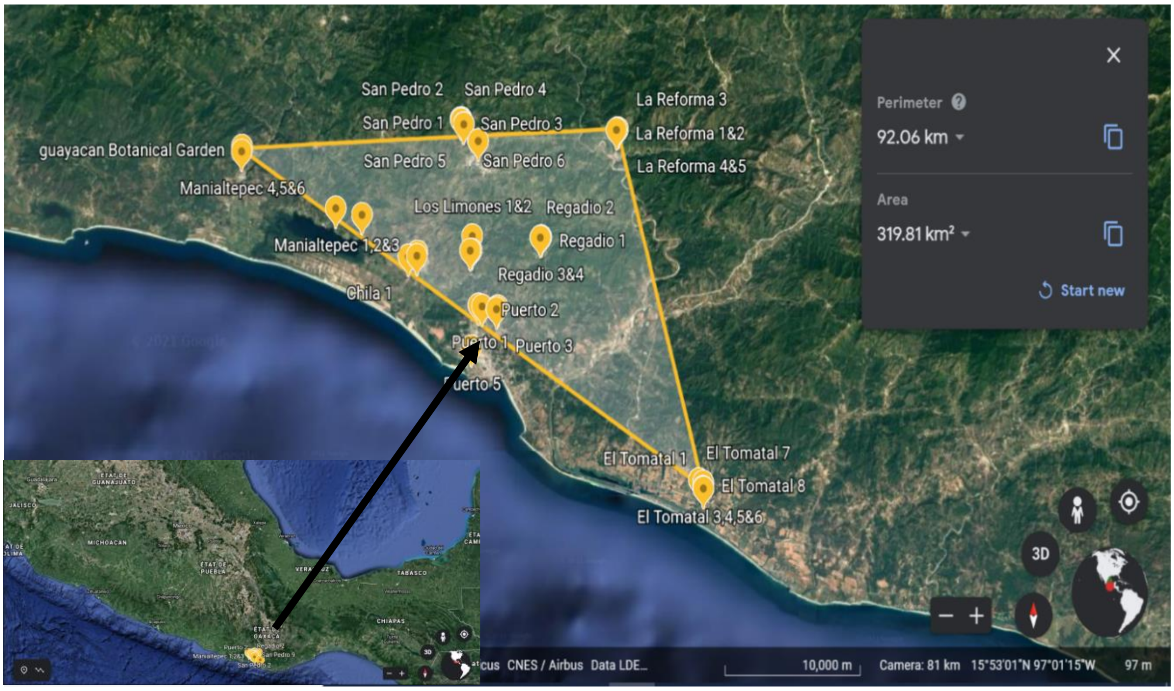


Fig. S5 Map of geographic locations of sampled wild chili accessions around Puerto Escondido (Oaxaca, Mexico) developed using Google Earth Pro (version 7.3).

4. Domestication of chili pepper has altered fruit traits affecting the oviposition and feeding behavior of the pepper weevil

Yosra Chabaane [1], Muhammad Haseeb [2] and Betty Benrey [1*]

[1]Laboratory of Evolutionary Entomology, University of Neuchâtel, 2000 Neuchâtel, Switzerland; yosra.chabaane@unine.ch

[2]Center for Biological Control, College of Agriculture and Food Sciences, Florida A&M University, Tallahassee, FL 32307-4100, USA; muhammad.haseeb@famu.edu

[*] Correspondence: betty.benrey@unine.ch ; Tel.: +41-32-718-31-32

Published in the Journal of Pest Science

12 July 2021

https://www.mdpi.com/2075-4450/12/7/630

https://doi.org/10.3390/insects12070630

Simple summary

The pepper weevil is an economically important pest that causes major damage to fruits of chili pepper varieties selected for consumption. However, the impact of this pest on wild and ornamental peppers remains unknown. Therefore, we studied the effect of chili domestication on the feeding and oviposition behavior of pepper weevil when exposed to wild chili, ornamental varieties, and varieties used for consumption. More specifically, we examined how changes in fruit and flower size, fruit thickness, spiciness level, and fruit position as a result of the domestication of chili peppers affected their susceptibility to this specialist pepper pest. In addition, we recorded that fruits and flowers from wild and ornamental plants were less susceptible to pepper weevil attacks than those from chili varieties selected for consumption. Our results have important implications for chili pepper breeders and could guide the selection of new resistant varieties against this pest.

Abstract

The pepper weevil, *Anthonomus eugenii*, Cano (Coleoptera: Curculionidae), is one of the most destructive pests of chili pepper. It causes extensive damage on varieties selected for consumption. However, the occurrence of this pest on wild and ornamental peppers remains unknown. We investigated the consequences of chili domestication on the feeding and oviposition of *A. eugenii* on fruits and flowers. We used plants of one wild accession, Bird Eye Pepper, five ornamental varieties (Pops Yellow, Black Pearl, Sedona Sun, Chilli Chilli, and Salsa Deep), and two domesticated varieties selected for consumption (Scotch Bonnet and Jalapeño). First, we characterized the plants according to their fruit and flower sizes, pericarp thickness, capsaicin level, fruit position, and flower color. Then, we evaluated the susceptibility of fruits and flowers to *A. eugenii*. Overall, domestication increased fruit and flower sizes and pericarp thickness, altered capsaicin levels, and altered fruit position and flower color. Weevils laid more eggs and caused more feeding damage on varieties selected for consumption than on wild and ornamental plants. Our results add to the growing literature on the consequences of crop domestication on herbivores. This knowledge could be integrated into breeding programs to select varieties resistant against the pepper weevil.

Keywords *Anthonomus eugenii*; oviposition; feeding behavior; chili domestication; plant traits; wild chilies

4.1. Introduction

The pepper weevil (*Anthonomus eugenii,* Cano) is a pepper (*Capsicum* spp.) specialist, although it is reported to attack other crops in the Solanaceae family such as eggplants (*Solanum melongena* L.) and the common black nightshade (*Solanum americanum* Mill.) [1,2]. It is a major pest of peppers in Mexico, its place of origin, as well as in the Caribbean and Southern United States, where it was first reported in Texas in 1904 [3–5]. Apart from its natural range, international trade has contributed to the spread of this pest in other countries (e.g., Canada, Netherlands, and Italy) [6–8]. Because of the widespread use of chili peppers in many countries [9], there is increasing concern that this pest could be inadvertently introduced worldwide. Females lay eggs singly in a cavity made with their rostrum (mouthpart), then seal the puncture with a brown fluid, secreted through the ovipositor, that hardens into a solid egg-plug (Video S1) [5,10]. Upon hatching, the larvae feed on the seeds and soft tissue inside the developing fruit, whereas the adults feed on the flowers and young fruits (Figure 1) [6]. Females lay the maximum (3.1 eggs/day) on flower buds and/or on small fruits [11]. Their life cycle consists of an egg stage, three larval stages, pupal stage completed entirely inside the fruits, followed by the adult stage when *A. eugenii* exits the fruit by making a hole with their rostrum [1,5,12]. The typical damage of this insect is small holes on immature fruits, causing fruit deformation and premature fruit ripening and dropping [13].

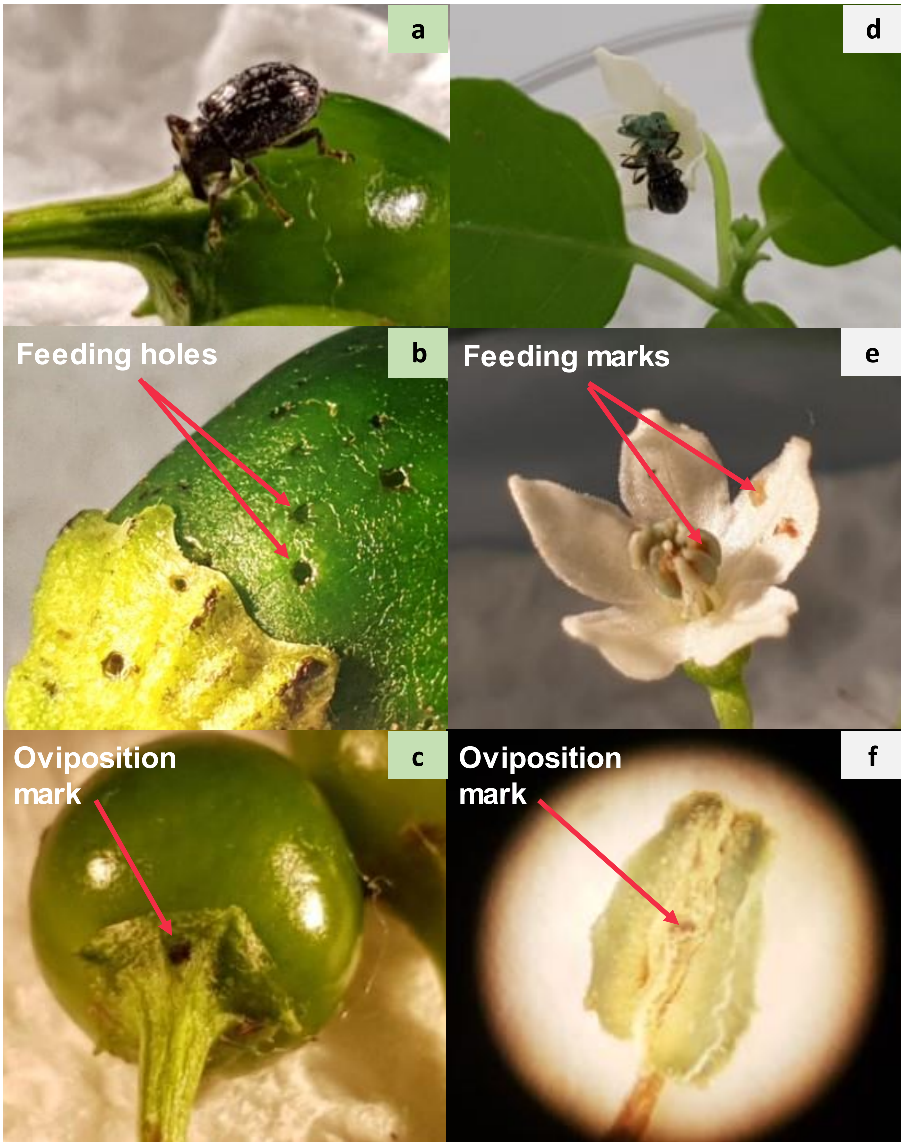


Fig.1 Pepper weevil damage on chili fruits (**a**, **b**, and **c**) and flowers (**d**, **e**, and **f**). All images © Y. Chabaane.

The use of insecticides to control *A. eugenii* could be ineffective once the population is established in the field, because the entire development occurs inside the fruit [10,14]. Therefore, pest scouting methods using yellow sticky traps with a pheromone are recommended to establish the timing of pesticide application and reduce the adult population [15,16]. The use of natural enemies could offer an environmentally safe control method for this pest. In a survey conducted in Mexico [17], thirteen different species of parasitoids were reported to attack the pepper weevil. The main species found was *Catolaccus hunteri* Crawford (Hymenoptera: Pteromalidae), an ectoparasitoid of third larval stages. Parasitoid larvae develop externally on the larval host, and after two weeks, the adult parasitoids emerge [18]. However, the establishment of these parasitoids in new areas where the pepper weevil is introduced remains a challenge [19]. Therefore, additional pest management strategies are needed to control this pest in the open fields. Host plant resistance is an effective, economical, and ecofriendly method for pest control [20,21]. In this context, Berdegue et al. [22] explored the plant resistance against the pepper weevil of 23 virus-resistant lines that belong to cultivars of various *C. anuum* varieties, including Jalapeño, bell, pimiento, serrano, yellow, cayenne, long chile, tabasco, and cherry. They found that synchronous fruit production reduced the period of susceptibility to attack by *A. eugenii.* In another study, Seal and Martin [3] found that the highly pungent Habanero variety (*C. chinense* Jacquin) was more resistant to pepper weevil than nonpungent or mild *C. annum* cultivars, including Bell, Hungarian wax, or Jalapeño peppers. This suggests that the pungency level in fruits could be involved in plant resistance against this pest. However, this idea remains to be tested.

The above studies used only chili varieties selected for fruit consumption. However, unlike many other crops, wild chili is also consumed either fresh or dry as condiment [23]. In Mexico and Southern US, wild chili plants grow in backyard gardens together with other domesticated plants (Chabaane, personal observation). In their natural habitat, wild chili provides an essential ecosystem service for local habitants who sell the dry fruits [24]. In addition to being domesticated for consumption, chili pepper has been selected for ornamental use. Ornamental peppers are morphologically diverse in fruit size, ripe fruit colors, foliar pigmentation that varies from green to purple, fruit and leaf shape [25]. In addition to their fruits, ornamental peppers were selected for different flower colors and size [26,27]. They were also selected for rapid seed propagation, heat, and drought tolerance [25,28,29]. When introduced to Europe in the 15th century, chili peppers were admired more as an ornamental plant than as a food source [25]. Today, they are gaining in popularity worldwide due to their beauty [29–32]. They were also reported as a potential alternative food source, known as a banker plant, for natural enemies of different pests such as the generalist mite *Amblyseius swirskii* or the insidious flower bug *Orius insidiosus* [33–36]. Despite the importance of wild and ornamental peppers, studies on the pepper weevil as a pest of chili peppers have essentially focused on varieties used for cooking. To date, there are no comparable studies that evaluate the impact of this pest on ornamental and wild chilies.

The suite of physiological and morphological traits that distinguish crops from their wild relatives is known as domestication syndrome [37]. For chili pepper, domestication has increased seed germination rate and fruit size, increased variation in fruit color, and changed fruit position from upright to pendant hidden by the foliage to reduce fruit predation by birds [38,39]. In addition, domestication influenced floral phenology, flower size and color, especially for varieties selected as ornamentals to make them more attractive [26,27]. The most characteristic trait of pepper is the capsaicinoids in fruits that are responsible for the spiciness or pungent taste [40]. Two capsaicinoids, capsaicin and dihydrocapsaicin, represent 90% of the whole capsaicinoids in fruits and are produced in the placenta [41]. In contrast to other crops for which domestication decreased secondary metabolites [42], species and varieties in the genus C*apsicum* have been selected for both increased and decreased levels of capsaicinoids [43,44] as compared to those of the wild ancestor, Chiltepin (*C. annuum* var. *glabriusculum*) [45,46]. A scale from low to high pungency (from 0 to 1,500,000 Scoville heat units = SHU), known as the Scoville chart, was developed to characterize chili varieties according to their spiciness [47]. According to the Scoville chart, Chiltepin has an intermediate pungency level

(100,000–250,000 SHU) between highly pungent varieties (e.g., Habanero) and mild varieties (e.g., Jalapeño).

The antifungal, antibacterial, andmedicinal uses of capsaicin are very well-studied [48–52]. However, their effects on insects are poorly known, although it is commonly used as a pesticide [53,54]. In a previous study, we found that capsaicin reduced larval development, pupation, and adult emergence of the generalist herbivore (*Spodoptera latifascia*, Walker) and reduced the parasitism rate of its ectoparasitoid (*Euplecterus platyhypenae*, Howard) [55]. However, the effect of capsaicin on the specialist herbivore (*A. eugenii*) remains unclear. Moreover, it is still not known how altered traits and the purpose of domestication (for consumption or as ornamental) have affected the interaction between chili fruits and the pepper weevil.

In this study, we investigated the consequences of chili domestication on the feeding and oviposition behavior of the pepper weevil. To do so, we used one wild accession called Bird Eye Pepper, five ornamental varieties (Pops Yellow, Black Pearl, Sedona Sun, Chilli Chilli, and Salsa Deep), and two domesticated varieties selected for fruit consumption, Scotch Bonnet and Jalapeño. Our specific objectives were: (1) to examine how domestication has altered fruit size and pericarp thickness, fruit position, pungency level, and flower size and color; (2) to determine how these altered traits affect the feeding and oviposition of the pepper weevil. The results from this study will allow us, first, to evaluate the impact of chili domestication on the performance of this specialist pest and secondly, to identify plant traits potentially responsible for resistance against this insect that could be integrated in further breeding programs.

4.2. Materials and methods

4.2.1. Plants

- **Wild Plants**

Wild fruits of the Bird Eye Pepper (*Capsicum annuum* L. var. *glabriusculum*) were provided by Dr. Erdwin (FAMU, Tallahassee, FL, USA). The peppers are very small and grow spontaneously in backyard gardens from where they are collected, dried, or pickled in vinegar and used in many dishes. The fruits have an upright position on the plant, as in Figure S1a. Although the *C. annum* pepper is originally from Mexico, the wild Chiltepin can also be found in Southern United States (Texas, Arizona, and Florida), where it is known as Bird Eye Pepper [56].

- **Ornamental Plants**

Plants were obtained from Tallahassee nursery https://www.tallahasseenurseries.com/ (accessed on 10 July 2021). We used the fruits of five ornamental varieties: Pops Yellow, Black Pearl, Sedona Sun, Chilli Chilli, and Salsa Deep. They differ in color, size, and form. They all have an upright position like the wild Bird Eye Pepper (Figure S1b–f). They all belong to the species *Capsicum annum*.

- **Domesticated Plants for Fruit Consumption**

We used three domesticated varieties that were selected for consumption and have been integrated into many cuisines, Jalapeño (*C. annum*), Pepperoncino (*C. annum*), and Scotch Bonnet (*C. chinense*). They were collected at the Florida A&M University (FAMU) Research and Extension Center in Quincy and by a farmer, Dr. Bravo Brown in Cairo, Georgia. Pepperoncino fruits were purchased from the market in Neuchâtel, Switzerland. Fruits from Jalapeño and Scotch Bonnet are pendant and hidden by foliage (Figure S1 h,i). However, Pepperoncino fruits have an upright position (Figure S1g). The experiment on fruit size using the variety Peperoncino was conducted in Neuchâtel, Switzerland. All other experiments were conducted in Florida, where the pepper weevil is a major pest on chili peppers in open fields and greenhouses and where these plants are extensively grown [57,58].

4.2.2. Insects

Jalapeño fruits with signs of *A. eugenii* infestation were collected from the field at the University of Georgia, Tifton Campus. Insects were kept inside rearing cages (30 cm × 30 cm × 30 cm) in an incubator at 28 ± 2 C, 70± 5 % RH, and 14:10 L:D period. The cages were checked daily for the collection of adults.

4.2.3. Fruit size and pericarp thickness measurements

Fruit length, width and height, and pericarp thickness measurements were taken with an electronic digital caliper (Vogel, Kevelaer, Germany) with a measuring range from 0 to 150 mm and a resolution of 0.01 mm. Fruit size was calculated as follows: Fruit size (cm3) = [Length (mm) × Width (mm) × Hight (mm)]/10

4.2.4. Capsaicin Level in Fruits

The capsaicin level in fruits was characterized according to the pungency level reported in the literature and was based on the Scoville chart (Scoville Heat Unit = SHU) [47,59–62] (Figure 2).

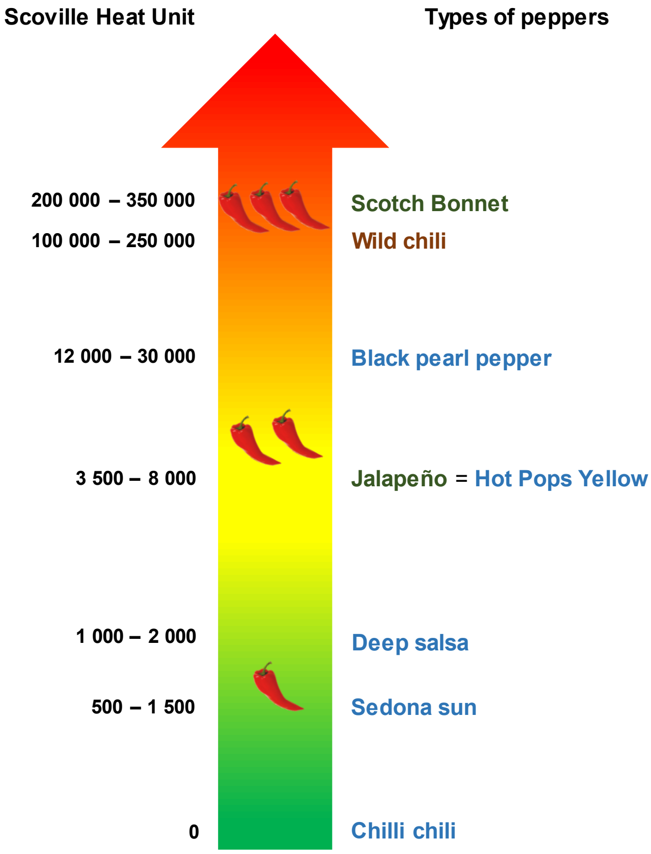


Fig. 2 Characterization of capsaicin level in wild (Brown), domesticated as ornamental (Blue), and domesticated for consumption (Green) peppers according to the Scoville scale. © Y. Chabaane.

4.2.5 Effect of domestication on fruit infestation by the pepper weevil

The aim of this experiment was to investigate whether the domestication status of chili fruits had an effect on the feeding and oviposition behavior of the pepper weevil. We conducted a no-choice test in Petri dishes (100 mm × 20 mm) with one wild accession (Bird Eye), five ornamental peppers (Pops Yellow, Black Pearl, Sedona Sun, Chilli Chilli, and Salsa Deep), and two varieties used for consumption (Jalapeño and Scotch Bonnet). We placed two fruits from each variety/accession with one couple of pepper weevils for one day. We had 10 replicates (Petri dishes) per treatment. After one day of infestation, we recorded the feeding holes and oviposition marks per fruit (a total of 20 fruits per treatment).

4.2.6. Effect of Fruit Size on the Infestation by the Pepper Weevil

To examine the effect of fruit size on feeding and oviposition, we conducted choice and no-choice tests with three categories of fruit size (small, medium, and large) with the same variety, Pepperoncino. For the choice test, we placed one fruit of each size category in the same Petri dish and for the no-choice test, we placed three fruits of the same size category in one Petri dish. We added one couple of weevils per Petri dish and counted the number of feeding holes and oviposition marks after one day of infestation. We had 10 replicates for each test (10 Petri dishes for the choice test and 30 Petri dishes for the no-choice test).

4.2.7. Effect of Domestication on Flower Infestation by Female Pepper Weevils

As female weevils feed and lay eggs not only on the fruits but also on the flowers, we quantified the infestation on wild and domesticated flowers. We used flowers from the wild accession (Bird Eye), the ornamental pepper (Black Pearl), and the Jalapeño variety. Pepper weevils are also known to infest buds and flowers of other Solanaceae, so we included, as a comparison, the flowers of eggplant (*Solanum melongena* L.) [63]. Flower size was estimated using photos and a scale of 10 mm to evaluate the differences among treatments. Flowers of the wild pepper and Jalapeño are white, and the ornamental Black Pearl and eggplant flowers are purple. To study the feeding and oviposition of female weevils on the different flowers, one female was placed in a Petri dish with one flower (100 mm × 20 mm). We had five replicates (Petri dishes) per treatment. Flower infestation was evaluated at two time periods. The first period was 20 min after the release of the insect, and we referred to this as early infestation. During this period, we recorded the searching time, which is the time allocated by the mated female to find and start exploring the flower as well as the percentage of infested flowers (i.e., flowers with feeding marks) [(infested flowers/total number of flowers) ×100]. The second period was after 24 h (late infestation), we recorded again the percentage of infested flowers and the number of eggs laid per flower.

4.2.8. Statistical Analysis

All statistical analyses were performed with R statistical software (version 3.5.3; R Development Core Team, 2020), using ANOVA, followed by residual analysis to verify the suitability of distributions of the tested models. Generalized Linear Mixed Models (GLMMs) with a Gaussian distribution, followed by post hoc analysis (Tukey's), were used to compare the data on fruit and flower sizes, pericarp thickness, feeding holes in fruits, number of eggs in fruits and flowers, searching time on flowers, and number of infested flowers. Fruits and Petri dishes were included as random factors. The overdispersion of the data was verified and when necessary, the correction quasibinomial was applied. The sample size and number of replicates for all experiments are indicated directly in the figure captions.

4.3. Results

4.3.1. Effect of chili domestication on the fruit size, pericarp thickness, and capsaicin level

- **Fruit Size**

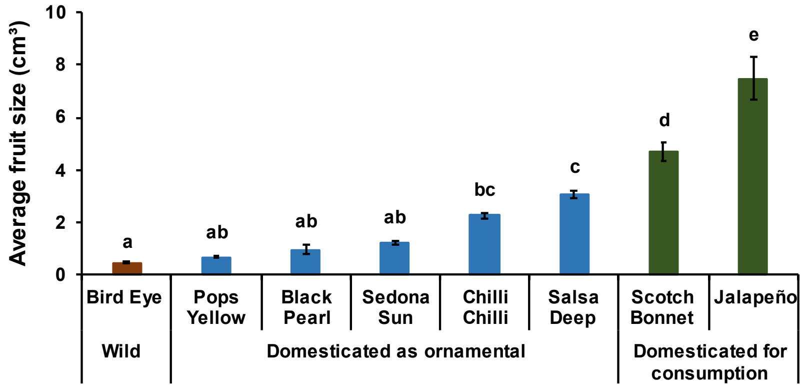
Overall, chili domestication increased fruit size (Figure 3a). Except for the three ornamental peppers, Pops Yellow, Black Pearl, and Sedona Sun, fruits of the other domesticated varieties were significantly larger than those of the wild Bird Eye Pepper (F[7,247] = 23.609, d.f = 7, p < 0.001). Fruits domesticated for consumption were at least 35% larger than ornamental fruits, and the largest fruit size was recorded for the Jalapeño variety.

- **Pericarp fruit thickness**

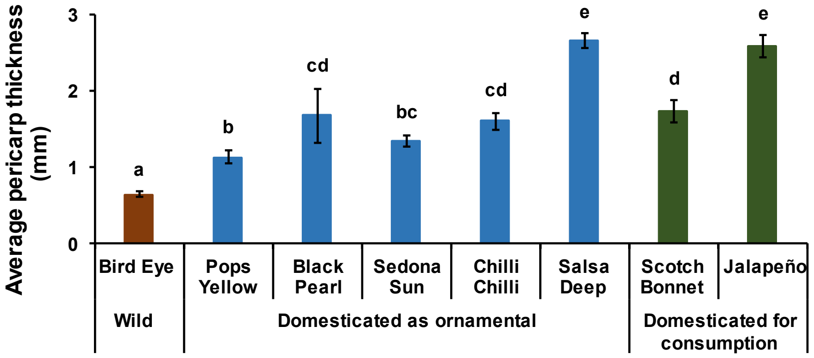
Chili domestication increased thickness (Figure 3b). All ornamental and consumption varieties had a thicker pericarp compared to that of the wild Bird Eye Pepper (F[7,247] = 30.279, d.f = 7, p < 0.001). The ornamental Salsa Deep Pepper and Jalapeño variety had the thickest pericarp, over 2.5 mm, which was three times more than the thickness recorded on wild fruits.

- **Capsaicin level in fruits**

Based on the pungency level reported in the literature and the Scoville chart (Figure 2), the characterization of chili pepper revealed that the pungency level for wild pepper (100,000–250,000 SHU) was intermediate, between those of the spicy Scotch Bonnet (200,000–350,000 SHU) and the mild Jalapeño (3500–8000 SHU), both varieties used for consumption. However, the capsaicin level in ornamental varieties was lower than in the wild pepper, within a gradient from 0 SHU (nonpungent Chilli Chilli variety) to around 30,000 SHU (Black Pearl variety).



(**a**)



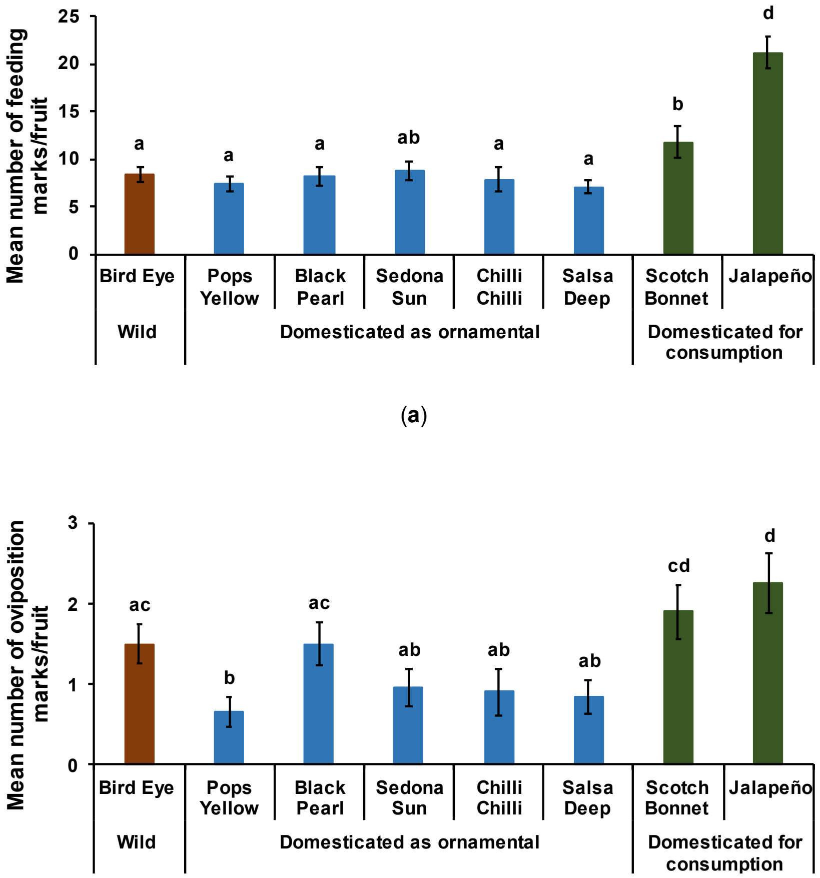
(**b**)

Fig. 3 Effect of chili domestication on fruit size (a) and pericarp thickness (b). Sample size: Bird (N= 25); Pops Yellow (N= 20); Black Pearl (N = 25); Sedona Sun (N = 30); Chilli Chilli (N = 20); Salsa Deep (N = 41); Scotch Bonnet (N = 32); Jalapeño (N = 62). The differences among treatments are indicated by different letters (F-test, Tukey's post-hoc test with Bonferroni correction: $p < 0.001$).

4.3.2. Effect of domestication on fruit infestation by the pepper weevil

- **Feeding on fruits**

Overall, both the wild and ornamental varieties were less attacked by the pepper weevil as compared to the varieties selected for consumption (Jalapeño and Scotch Bonnet) (Figure 4a; F[7,152] = 15.809, d.f = 7, $p < 0.001$). The only exception was Sedona Sun (ornamental) that had as many feeding marks (around 10 marks per fruit) when compared to Scotch Bonnet. Moreover, the pepper weevil fed 45% more on Jalapeño than on Scotch Bonnet. We did not find significant differences for the feeding marks between wild pepper and ornamental varieties.



(a)

(b)

Fig. 4 Infestation by pepper weevil on ornamental and domesticated peppers showing the mean number of feeding per fruit (a) and the oviposition marks per fruit (b). No choice tests using two fruits per Petri dish. Difference among treatments is indicated by different letters (F-test, Tukey post-hoc test with Bonferroni correction: P<0.001, N= 20).

- **Oviposition on fruits**

Female weevils laid 34% more eggs on varieties used for consumption (Jalapeño and Scotch Bonnet) than on wild pepper or ornamental varieties (Figure 4b; F[7,152] = 4.3715, d.f = 7, p < 0.001). However, Bird Eye Pepper and the ornamental variety Black Pearl had as many oviposition marks as Scotch Bonnet, but significantly fewer than Jalapeño. Varieties used for the same purpose of domestication (ornamental or consumption) had no significant differences for the number of eggs laid per fruit.

4.3.3. Effect of fruit size on the infestation by the pepper weevil

- **Choice test**

We found significant differences in fruit size for the three categories, small, medium, and large, shown in (Figure 5a; F[2,27] = 151.94, d.f = 2, p < 0.001). We did not find significant effect of fruit size on feeding marks (Figure 5b; F[2,27] = 0.3631, d.f = 2, p = 0.6989). However, females laid more eggs on small fruits than on large or medium fruits (Figure 5c; F[2,27] = 13.968, d.f = 2, p < 0.001).

- **No-choice test**

The three categories used for this test had significantly different fruit size (Figure 5d; F[2,87] = 273.28, d.f = 2, p < 0.001). We did not find significant effects of fruit size on feeding (Figure 5e; F[2,87] = 0.0583, d.f = 2, p = 0.9434) or oviposition marks by the pepper weevils (Figure 5f; F[2,87] = 1.448, d.f = 2, p = 0.2416).

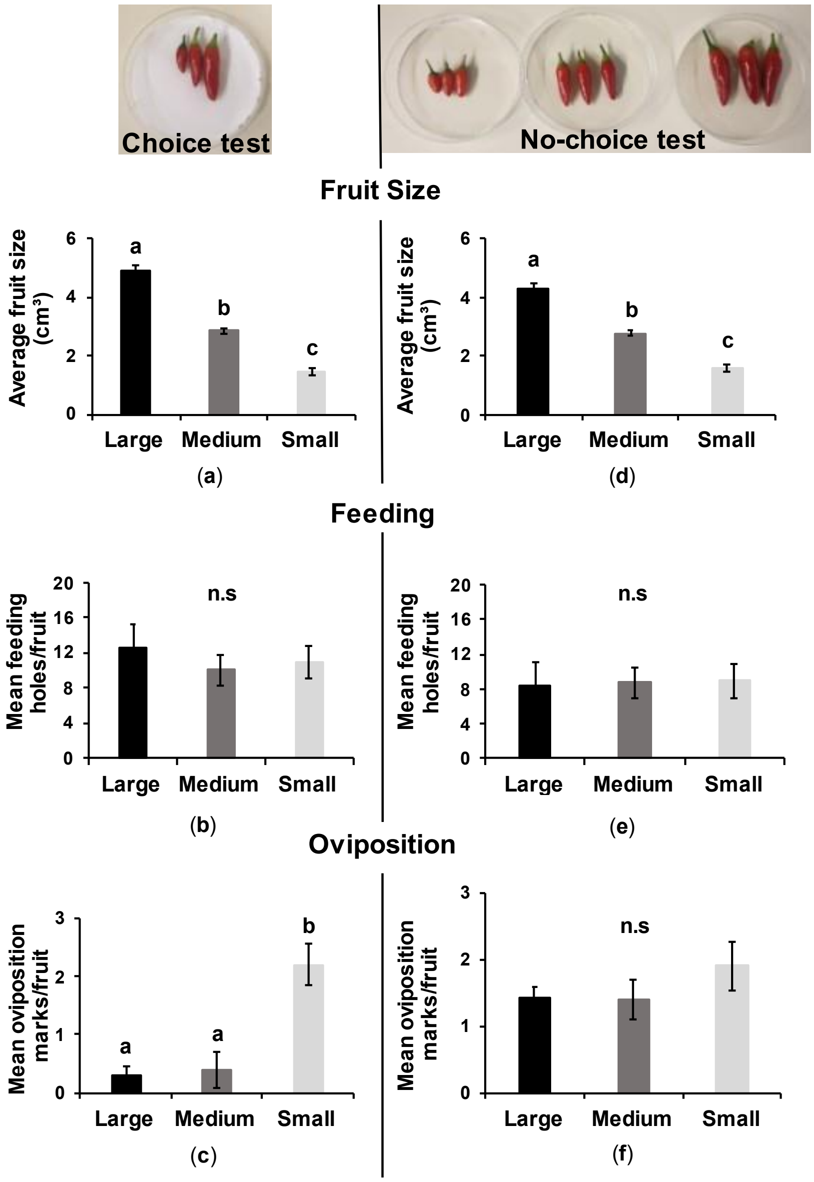


Fig. 5 Effect of fruit size on the feeding and oviposition of the pepper weevil on pepperoncino variety, using choice (a, b and c) and no choice tests (d, e and f). The difference among treatments is indicated by different letters (F-test, Tukey post-hoc test with Bonferroni correction: P<0.01, N= 10). All images © Y. Chabaane.

4.3.4. Effect of domestication on flower infestation by female pepper weevils

- **Flower size**

We found significant differences in flower size among the four treatments (F[3,16] = 95.667, d.f = 3, p < 0.001). Flowers of the wild Bird Eye were the smallest (15.8 0.9 mm), followed by the ornamental Black Pearl (43.6 3.2 mm) and the variety selected for consumption, Jalapeño (73.4 2.9 mm), and the largest flowers were from the eggplants (121.4 8 mm).

- **Searching time**

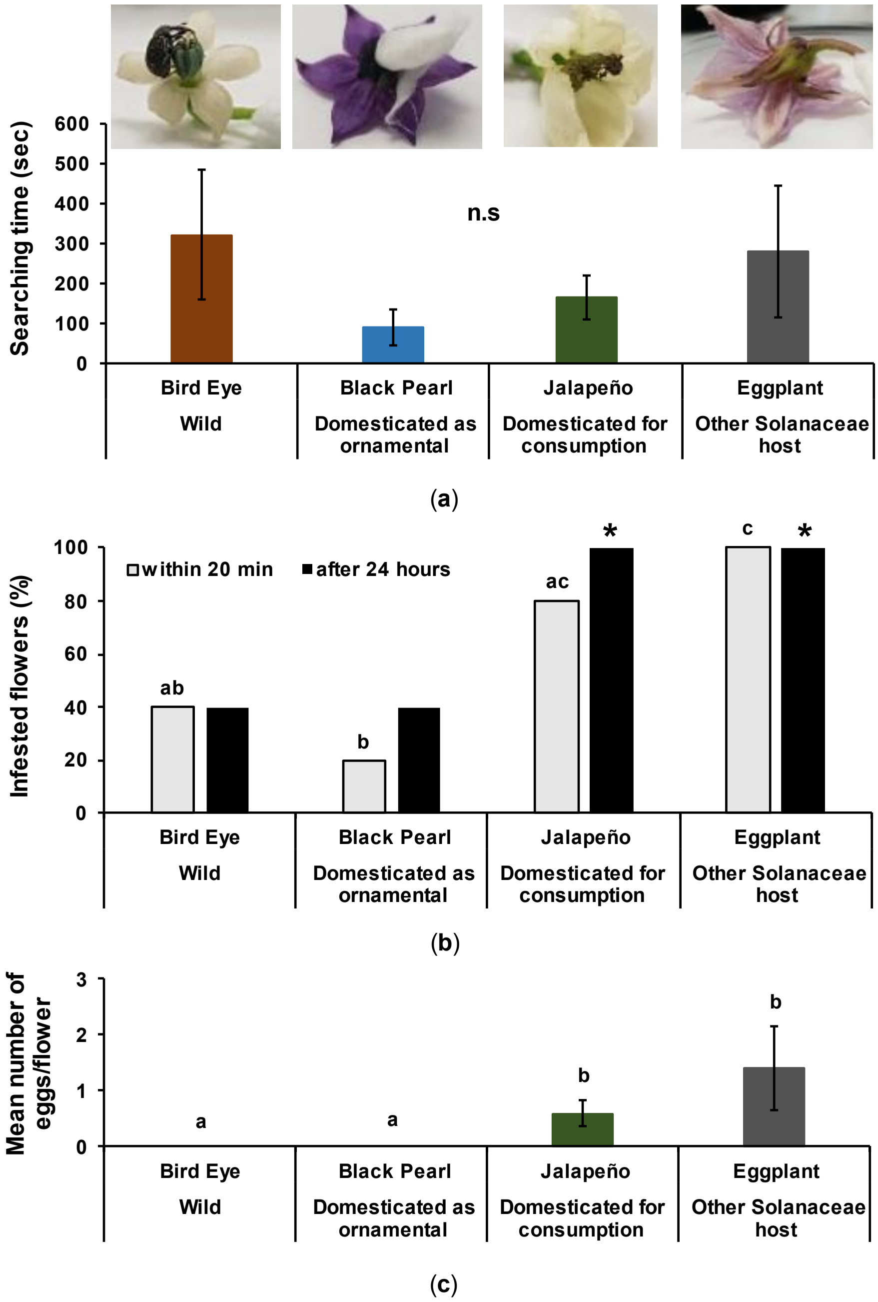
We did not find significant differences for the time spent by pepper weevils to find the flowers (Figure 6a; F[3,16] = 43.891, d.f = 3, p = 0.7018). (Figure 6a; F[3,16] = 43.891, d.f = 3, p = 0.7018).

- **Infestation on flowers**

Early (after 20 min) and late infestation (after 24 h) on flowers are shown in Figure 6b. Within the first 20 min of the experiment, the infestation was 40% to 60% higher on the Jalapeño flowers than on wild and ornamental flowers. The eggplant flowers were all infested within 20 min (Figure 6b; χ2 = 16.738, d.f = 3, p = 0.01708 *). This effect was further confirmed after 24 h (Figure 6b; χ2 = 13.460, d.f = 3, p = 0.01187 *). The weevils attacked the wild (Bird Eye) varieties more than the ornamental flowers (Black Pearl) at the beginning of the experiment, but the infestation level was similar after one day.

- **Oviposition on flowers**

We found significant differences for the number of eggs on flowers (Figure 6c; F[3,16] = 1.22, d.f = 3, p = 0.01562 *). Female weevils laid eggs only on Jalapeño and eggplant flowers, and none on wild and ornamental flowers.



(a)

(b)

(c)

Fig. 6 Pepper weevil attack on flowers of wild pepper (Bird Eye), ornamental pepper (Black Pearl), domesticated pepper for consumption (Jalapeño), and Eggplant. Searching time to find the flower (a), percentage of infested flowers (b) and mean number of eggs per flower (c). Difference among treatments is indicated by different letters for 20 min and stars for after 24 h (F-test (a, c) and Chi-test (b) followed by Tukey post-hoc test with Bonferroni correction: $P<0.05$, N= 5). All images © Y. Chabaane.

4.4. Discussion

Pepper weevil causes considerable damage to domesticated chili varieties used for consumption. The present study was therefore aimed at investigating the unknown effects of this pest on ornamental and wild peppers. Overall, we found that chili domestication has increased fruit size and pericarp thickness, altered capsaicin levels by selecting varieties with lower and higher capsaicin content than the wild chili, and changed the fruit position of varieties used for consumption. Additionally, we found that weevils preferentially fed and oviposited on varieties selected for consumption. Thus, our results revealed that domesticated traits in varieties used for consumption of their fruits have shown increased susceptibility to pepper weevil.

Several studies have shown that domestication has increased the susceptibility of crops to herbivore attack [64–67]. In the case of chili pepper, different physical traits were reported to play an important role in plant resistance against the pepper weevil. For example, Wu, Haseeb [57] showed that *A. eugenii* preferred to feed and oviposit on small sized, thin-walled, and small-mass fruits over large-sized, thick-walled, and large-mass fruits from the same variety. In a choice test, we found similar results across varieties females preferred to oviposit on small rather than medium-size or large fruit varieties, but not within the same variety; weevils fed equally on Pepperoncino fruits regardless of their size. Seal and Martin [3] also found that when offered different cultivars, weevils were more attracted to medium to large fruits (>1.5 cm long) than to small fruits (<1.5 cm).

Our results showed that Jalapeño and Scotch Bonnet, both consumption varieties, had the largest fruit size and were attacked by weevils the most compared to ornamental and wild peppers with smaller fruits. A plausible explanation is that beetles choose cultivars with large fruits that offer more resources for feeding and oviposition and possibly a lower risk of predation [68]. Conversely, Porter, Lewis [69] found that within the same variety (Jalapeño), weevils preferred small fruits. They suggest that small fruits resemble flower buds, where the first infestations take place [69]. Although we only had two varieties selected for consumption, chili fruits domesticated for this purpose are known to be larger than wild and ornamental fruits [25,70–72]. Indeed, Silva, Jasmim [70] point out that in the production of ornamental plants, breeders tend to favor small-sized fruits present in large quantities, traits that enhance their attractiveness and beauty.

Pericarp thickness has also been proposed as a plant trait involved in resistance against this pest [73]. In cotton, the boll wall thickness was responsible for reduced damage on cotton plants caused by the boll weevil (*Anthonomus grandis grandis*, Boheman), a sister species of the pepper weevil [74]. In our study, although domestication increased pericarp thickness, this did not seem to influence fruit susceptibility to pepper weevil, as varieties domesticated for consumption suffered the most damage. Moreover, most of the ornamental varieties had a pericarp as thick as that of Scotch Bonnet and Jalapeño fruits, but they were less damaged by the weevils. This suggests that other traits (e.g., fruit size) must be responsible for the increased susceptibility of consumption varieties. In addition, the maximum pericarp thickness recorded in our study was around 2.6 mm (Jalapeño and Salsa Deep varieties). This measurement is doubled and may reach over 6 mm for some bell pepper cultivars [75] and may thus explain their resistance to the pepper weevil [73]. The female weevil creates a cavity with her rostrum (with a mandibulated mouthpart) before depositing the egg and seals the puncture with a brown fluid that hardens and darkens into a solid egg-plug (Video S1) [1,5,10]. The rostrum length for both sexes is around 1.5 mm (Figure S2). Therefore, a thick pericarp may hinder the feeding and oviposition of this insect. Future breeding programs could exploit this knowledge and select varieties with thicker pericarps. However, selecting varieties with a thicker pericarp may not always be compatible with the use of natural enemies for biological control. For example, the ability of the parasitoid *C. hunteri* to insert its ovipositor and parasitize the larvae of the pepper weevil decreases in fruits with a thicker pericarp [76]. Fruit position is another plant trait that was targeted by domestication [38,40]. The shift was from an upright position for wild chilies to pendant, hidden by foliage for many but not all domesticated plants [40]. For example, the fruits of the ornamental varieties used in our study kept the upright position similar to that of the wild Bird Eye (Figure S1). Therefore, we expect that these plants could be more exposed to insect and bird attacks compared to other varieties where fruits are pendant and hidden under leaves (e.g., Jalapeño and Scotch Bonnet). This hypothesis remains to be tested.

In contrast to other crops for which domestication aimed at reducing secondary metabolites [42], chili pepper was selected for both increased and decreased levels of capsaicinoids [43,44] as compared to that in the wild ancestor, Chiltepin [45,46]. Because in the current study we used only one wild chili accession, we should be cautious with our conclusions pertaining to the wild chili. However, in another study with nine Chiltepin populations collected in Southern Mexico, we found minimal variation in capsaicin levels among populations (Chabaane et al., unpublished data). Conversely, capsaicin levels vary greatly among the domesticated varieties (Figure 2) [47,55]. In insects, some studies with dried chili fruits and capsaicin-spiked artificial diet have demonstrated that capsaicin inhibits the feeding of *Henosepilachna vigintioctomaculata*, Motschulsky [77], deters oviposition of *Delia antiqua*, Meigen [78], and slows down larval development of *Earias insulana*, Boisduval [79]. The effects of capsaicin on generalist and specialist herbivores are expected to be different. For example, Ahn, Badenes-Pérez [80] reported that feeding on a capsaicin-spiked diet slowed the development of the generalist herbivores *Spodoptera frugiperda* JE Smith, *Heliothis virescens* Fabricius, and *Helicoverpa zea* Boddie, but did not affect the growth and survival of larvae of the tobacco budworm (*Helicoverpa assulta*), a specialist on Solanaceae. The latter can detoxify these secondary metabolites when feeding on hot pepper fruits [81,82]. In a previous study with fresh chili fruits, we also found that capsaicin had a negative effect not only on the generalist herbivore (*Spodoptera latifascia*, Walker) but also on its ectoparasitoid (*Euplecterus platyhypenae*, Howard), only at high doses [55].

Our results showed that the selection of fruits by the weevils based on capsaicin levels is unclear. Despite being a specialist, *A. eugenii* preferred to feed on and laid more eggs on Jalapeño fruits (mild) than on spicy peppers (e.g., Scotch Bonnet, Wild Bird Eye, and the ornamental Black Pearl). This preference for less pungent varieties was also reported by Seal and Martin [3]. They found that the highly pungent Habanero variety (*C. chinense* Jacquin) was less susceptible to *A. eugenii* than non-pungent or mild C. annum cultivars, including Bell and Jalapeño peppers [47]. However, in our study, both larvae and adults were also observed feeding on the fruit's placenta of the Scotch Bonnet variety (Chabaane, personal observation), where capsaicinoids are concentrated [83]. Meanwhile, the ornamental hot pops yellow fruit, mild in pungency, and the non-pungent ornamental Chilli Chilli were more resistant to the weevil's attack than the spicier chili varieties used for consumption (Figure 4a,b). As a specialist on chili pepper, it is expected that this pest is adapted to capsaicinoids, and both larvae and adults can tolerate the spiciness, but the mechanism remains unclear. We believe that it is more likely that other plant traits function as a defense against the pepper weevil.

For flowers, chili domestication increased the size and increased color variation, particularly for varieties selected as ornamentals, to make them more attractive for a commercial purpose [27]. We found that female weevils infested the larger flowers of Jalapeño and eggplant more. In contrast, flower color did not appear to have any effect on weevil infestation and oviposition. However, we should be cautious with this conclusion as we only used flowers from one representative of each category (wild, ornamental and for consumption, and eggplant as a comparison). In this study, we mainly focused on the consequences of chili domestication on morphological traits and capsaicin content, a direct chemical defense. Other traits like volatiles may also play a role in the host plant choice of the pepper weevil. Indeed, Addesso and McAuslane [63] showed that *A. eugenii* could detect and orient to constitutive host plant volatiles released from chili pepper fruits and flowers. Moreover, female weevils were attracted to damaged fruiting and flowering plants over undamaged plants, and preferred flowering and fruiting plants with actively feeding weevils over plants with old feeding damage [84]. Our results showed that both fruits and flowers of varieties used for consumption were more susceptible to these beetles than the ones from ornamental or wild plants. The extent to which fruit and flower volatiles play a role in this choice and whether domestication has altered these traits could be the subject of future research.

4.5. Conclusions

This study showed that chili domestication has altered morphological and chemical (capsaicin) traits in fruits with direct consequences for the feeding and oviposition by the pepper weevil. We focused on fruits and flowers mainly from the C. annum species (except for the Scotch Bonnet variety). Overall, we found that chili domestication increased fruit size and pericarp thickness, altered capsaicin levels, and changed the fruit position of varieties used for consumption. In addition, our results revealed that domesticated traits in varieties used for consumption have increased the susceptibility towards the pepper weevil. Accordingly, weevils preferred to feed on and laid more eggs on these varieties. Future work should examine other Capsicum species as well as additional plant traits (e.g., plant size, branching pattern, and volatile emissions) that might enhance resistance against this insect pest. For example, planting combinations of different chili varieties, including ornamental varieties and even wild chili plants, could create associational effects and reduce herbivory and disease transmission. A better understanding of the natural resistant traits of wild chili plants that have been altered as a result of domestication could help in the development of new varieties resistant to the pepper weevil and other pests.

Author contributions

Conceptualization, B.B. and Y.C.; methodology, Y.C. and B.B.; formal analysis, Y.C.; data curation, Y.C.; writing—original draft preparation, Y.C. and B.B.; writing—review and editing, Y.C., B.B. and M.H.; supervision, B.B. and M.H. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

We thank Davis Riley (University of Georgia) and Bravo Brown (Georgia pepper grower) for providing infested chili fruits to start the colony of the pepper weevil. We also thank Edwin Duke from (FAMU) and Anneita Gilbourn for providing wild pepper fruits. We are grateful to Jermaine Perier, Alberta Parkins, Donna Arnold, Warrel Diedrick, Elan Miles, and Halimah Wynn for their help with collecting insects and fruits in the field, taking care of plants, and assisting with the bioassays.

References

1.Capinera, J.L. Pepper Weevil, *Anthonomus eugenii* Cano (Insecta: Coleoptera: Curculionidae); University of Florida: Gainesville, FL, USA, 2004.

2. Patrock, R.; Schuster, D. Feeding, oviposition and development of the pepper weevil, (*Anthonomus eugenii* Cano), on selected species of Solanaceae. Int. J. Pest Manag. 1992, 38, 65–69.

3. Seal, D.R.; Martin, C.G. Pepper weevil (Coleoptera: Curculionidae) preferences for specific pepper cultivars, plant parts, fruit colors, fruit sizes, and timing. Insects 2016, 7, 9. [CrossRef] [PubMed]

4. Abreu, E.; Cruz, C. The occurrence of the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) in Puerto Rico. J. Agric. Univ. Puerto Rico 1985, 69, 223–224. [CrossRef]

5. Elmore, J.C.; Davis, A.C.; Campbell, R.E. The Pepper Weevil; United States Department of Agriculture: Washington, DC, USA, 1934.

6. Costello, R.; Gillespie, D. The pepper weevil, *Anthonomus eugenii* Cano as a greenhouse pest in Canada. Pepper Weevil 1993, 16, 31–34.

7. van der Gaag, D.J.; Schenk, M.; Loomans, A.; Delbianco, A.; Vos, S. Pest survey card on *Anthonomus eugenii*. EFSA Support. Publ. 2020, 17, 1887E.

8. Speranza, S.; Colonnelli, E.; Garonna, A.P.; Laudonia, S. First record of *Anthonomus eugenii* (Coleoptera: Curculionidae) in Italy. Fla. Entomol. 2014, 97, 844–845. [CrossRef]

9. FAOSTAT. Global Production of Vegetables in 2018, by Type. Available online: https://www.statista.com/statistics/264065 /global-production-of-vegetables-by-type/ (accessed on 12 January 2021).

10. Elmore, J.C.; Campbell, R.E. Control of the PepperWeevil. J. Econ. Entomol. 1954, 47, 1141–1143. [CrossRef]

11. Toapanta, M.A.; Schuster, D.J.; Stansly, P.A. Development and Life History of *Anthonomus eugenii* (Coleoptera: Curculionidae) at Constant Temperatures. Environ. Entomol. 2005, 34, 999–1008. [CrossRef]

12. Riley, D.; Sparks, A.N., Jr. The Pepper Weevil and Its Management; Texas Agricultural Extension Service: College Station, TX, USA, 1995.

13. Capinera, J. Pepper Weevil, *Anthonomus Eugenii* Cano, and Cuban Pepper Weevil, Fausfinus Cubae (Boheman) (Insecta: Coleoptera: Curculionidae); # EENY278; University of Florida EDIS Publication: Gainesville, FL, USA, 2014; Available online: http://edis.ifas. ufl.edu/in555 (accessed on 21 April 2019).

14. Seal, D.; Schuster, D. Control of Pepper Weevil, *Anthonomus eugenii* in West-Central and South Florida. Proc. Fla. State Hortic. Soc. 1995, 108, 220–224.

15. Riley, D.; Schuster, D.; Barfield, C. Refined action threshold for pepper weevil adults (Coleoptera: Curculionidae) in bell peppers. J. Econ. Entomol. 1992, 85, 1919–1925. [CrossRef]

16. Cartwright, B.; Teague, T.G.; Chandler, L.D.; Edelson, J.V.; Bentsen, G. An Action Threshold for Management of the Pepper Weevil (Coleoptera: Curculionidae) on Bell Peppers. J. Econ. Entomol. 1990, 83, 2003–2007. [CrossRef]

17. Rodríguez-Leyva, E.; Stansly, P.A.; Schuster, D.J.; Bravo-Mosqueda, E. Diversity and distribution of parasitoids of *Anthonomus eugenii* (Coleoptera: Curculionidae) from Mexico and prospects for biological control. Fla. Entomol. 2007, 90, 693–702. [CrossRef]

18. Vásquez, E.; Dean, D.; Schuster, D.; Van Etten, P. A laboratory method for rearing *Catolaccus hunteri* (Hymenoptera: Pteromalidae), a parasitoid of the pepper weevil (Coleoptera: Curculionidae). Fla. Entomol. 2005, 88, 191–194. [CrossRef]

19. Labbé, R.; Hilker, R.; Gagnier, D.; McCreary, C.; Gibson, G.; Fernandez-Triana, J.; Mason, P.; Gariepy, T. Natural enemies of *Anthonomus eugenii* (Coleoptera: Curculionidae) in Canada. Can. Entomol. 2018, 150, 404. [CrossRef]

20. Sharma, H.; Ortiz, R. Host plant resistance to insects: An eco-friendly approach for pest management and environment conservation. J. Environ. Biol. 2002, 23, 111–135. [PubMed]

21. Stout, M.J. Host-plant resistance in pest management. In Integrated Pest Management; Elsevier: Amsterdam, The Netherlands, 2014; pp. 1–21. [CrossRef]

22. Berdegue, M.; Harris, M.; Riley, D.; Villalon, B. Host Plant Resistance on Pepper to the PepperWeevil, 1991. Arthropod Manag. Tests 1995, 20, 365. [CrossRef]

23. Villalon-Mendoza, H.; Ramirez-Meraz, M.; Garza-Ocanas, F.; Maiti, R. Value Chain of Chile Piquin Wild Chili (*Capsicum annuum* L. var. *glabriusculum*) from Northeastern Mexico. Int. J. Bio-Resour. Stress Manag. 2016, 7. [CrossRef]

24. Egerer, M.H.; Fricke, E.C.; Rogers, H.S. Seed dispersal as an ecosystem service: Frugivore loss leads to decline of a socially valued plant, Capsicum frutescens. Ecol. Appl. 2018, 28, 655–667. [CrossRef]

25. Stommel, J.R.; Bosland, P.W. Ornamental pepper. In Flower Breeding and Genetics; Springer: Berlin/Heidelberg, Germany, 2007; pp. 561–599. [CrossRef]

26. Nascimento, N.; Nascimento, M.; Santos, R.; Bruckner, C.; Finger, F.; Rêgo, E.; Rêgo, M. Flower color variability in double and three-way hybrids of ornamental peppers. Acta Hortic. 2013, 1000, 457–464. [CrossRef]

27. Santos, R.; Nascimento, N.; Borém, A.; Finger, F.; Carvalho, G.; Nascimento, M.; Lemos, R.; Rêgo, E.; Rêgo, M. Ornamental pepper breeding: Could a chili be a flower ornamental plant? Acta Hortic. 2013, 451–456. [CrossRef]

28. Stommel, J.; Bosland, P. Pepper, ornamental, *Capsicum annuum*. In Flower Breeding and Genetics: Issues, Challenges and Opportunities for the 21st Century; Springer: Dordrecht, The Netherlands, 2006; pp. 561–599. [CrossRef]

29. do Rêgo, E.R.; do Rêgo, M.M. Ornamental pepper. Ornam. Crops 2018. [CrossRef]

30. Ari, E.; Yildirim, T.; Mutlu, N.; Büyükalaca, S.; Gökmen, Ü.; Akman, E. Comparison of different androgenesis protocols for doubled haploid plant production in ornamental pepper (*Capsicum annuum* L.). Turk. J. Biol. 2016, 40, 944–954. [CrossRef]

31. Norbert, S. Diversity of colour and shape in paprika and ornamental pepper Capsicum annuum L. in Hungary. In Proceedings of the National Pepper Conference, Naples, FL, USA, 8–11 December 1996; American Society for Horticultural Science: Alexandria, VA, USA, 1996.

32. Guo, Y.; Bai, J.; Duan, X.; Wang, J. Accumulation characteristics of carotenoids and adaptive fruit color variation in ornamental pepper. Sci. Hortic. 2021, 275, 109699. [CrossRef]

33. Avery, P.B.; Kumar, V.; Xiao, Y.; Powell, C.A.; McKenzie, C.L.; Osborne, L.S. Selecting an ornamental pepper banker plant for *Amblyseius swirskii* in floriculture crops. Arthropod-Plant Interact. 2014, 8, 49–56. [CrossRef]

34. Kumar, V.; Wekesa, V.W.; Avery, P.B.; Powell, C.A.; McKenzie, C.L.; Osborne, L.S. Effect of pollens of various ornamental pepper

cultivars on the development and reproduction of *Amblyseius swirskii* (Acari: Phytoseiidae). Fla. Entomol. 2014, 97, 367–373. [CrossRef]

35. Wong, S.K.; Frank, S.D. Influence of banker plants and spiders on biological control by *Orius insidiosus* (Heteroptera: Anthocoridae). Biol. Control 2012, 63, 181–187. [CrossRef]

36. Waite, M.O.; Scott-Dupree, C.D.; Brownbridge, M.; Buitenhuis, R.; Murphy, G. Evaluation of seven plant species/cultivars for their suitability as banker plants for Orius insidiosus (Say). BioControl 2014, 59, 79–87. [CrossRef]

37. Hammer, K. Das domestikationssyndrom. Die Kult. 1984, 32, 11–34. [CrossRef]

38. Pickersgill, B. Domestication of Plants in the Americas: Insights from Mendelian and Molecular Genetics. Ann. Bot. 2007, 100, 925–940. [CrossRef]

39. Ladizinsky, G. Plant Evolution under Domestication; Springer Science & Business Media: New York, NY, USA, 2012. [CrossRef]

40. Pickersgill, B. Chile peppers (Capsicum spp.). In Ethnobotany of Mexico; Springer: Berlin/Heidelberg, Germany, 2016; pp. 417–437. [CrossRef]

41. Govindarajan, V.; Salzer, U.J. Capsicum-production, technology, chemistry, and quality part 1: History, botany, cultivation, and primary processing. Crit. Rev. Food Sci. Nutr. 1985, 22, 109–176. [CrossRef]

42. Meyer, R.S.; DuVal, A.E.; Jensen, H.R. Patterns and processes in crop domestication: An historical review and quantitative analysis of 203 global food crops. New Phytol. 2012, 196, 29–48. [CrossRef]

43. Aza-González, C.; Núñez-Palenius, H.G.; Ochoa-Alejo, N. Molecular biology of capsaicinoid biosynthesis in chili pepper (*Capsicum* spp.). Plant Cell Rep. 2011, 30, 695–706. [CrossRef]

44. Kim, S.; Park, M.; Yeom, S.-I.; Kim, Y.-M.; Lee, J.M.; Lee, H.-A.; Seo, E.; Choi, J.; Cheong, K.; Kim, K.-T. Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat. Genet. 2014, 46, 270–278. [CrossRef] [PubMed]

45. González-Zamora, A.; Sierra-Campos, E.; Pérez-Morales, R.; Vázquez-Vázquez, C.; Gallegos-Robles, M.A.; López-Martínez, J.D.; García-Hernández, J.L. Measurement of capsaicinoids in chiltepin hot pepper: A comparison study between spectrophotometric method and high-performance liquid chromatography analysis. J. Chem. 2015, 2015, 709150. [CrossRef]

46. Tewksbury, J.J.; Reagan, K.M.; Machnicki, N.J.; Carlo, T.A.; Haak, D.C.; Peñaloza, A.L.C.; Levey, D.J. Evolutionary ecology of pungency in wild chilies. Proc. Natl. Acad. Sci. USA 2008, 105, 11808–11811. [CrossRef] [PubMed]

47. Scoville,W.L. Note on capsicums. J. Am. Pharm. Assoc. 1912, 1, 453–454. [CrossRef]

48. Marini, E.; Magi, G.; Mingoia, M.; Pugnaloni, A.; Facinelli, B. Antimicrobial and anti-virulence activity of capsaicin against erythromycin-resistant, cell-invasive group a *streptococci*. Front. Microbiol. 2015, 6, 1281. [CrossRef] [PubMed]

49. Argaez, L.G.B.; Salazar, F.F.M.; Zuñiga, F.Z.; Estrada, T.G.; Flores, I.R.I. Characterization of a *Capsicum chinense* seed peptide fraction with broad antibacterial activity. Asian J. Biochem. 2009, 4, 77–87. [CrossRef]

50. Omolo, M.A.;Wong, Z.-Z.; Mergen, K.; Hastings, J.C.; Le, N.C.; Reil, H.A.; Case, K.A.; Baumler, D.J. Antimicrobial properties of chili peppers. J. Infect. Dis. Ther. 2014, 2, 145. [CrossRef]

51. Haanpää, M.; Treede, R.-D. Capsaicin for neuropathic pain: Linking traditional medicine and molecular biology. Eur. Neurol. 2012, 68, 264–275. [CrossRef]

52. Witting, N.; Svensson, P.; Gottrup, H.; Arendt-Nielsen, L.; Jensen, T.S. Intramuscular and intradermal injection of capsaicin: A comparison of local and referred pain. PAIN® 2000, 84, 407–412. [CrossRef]

53. Koleva-Gudeva, L.; Mitrev, S.; Maksimova, V.; Spasov, D. Content of capsaicin extracted from hot pepper (*Capsicum annuum* ssp. microcarpum L.) and its use as an ecopesticide. Hem. Ind. 2013, 67, 671–675. [CrossRef]

54. Wilson, W.Wax and capsaicin based pesticide. J. Clean. Prod. 1996, 1, 61.

55. Chabaane, Y.; Arce, C.C.M.; Glauser, G.; Benrey, B. Altered Capsaicin Levels in Domesticated Chili Pepper Varieties Affect the Interaction Between a Generalist Herbivore and Its Ectoparasitoid. Res. Square 2021. [CrossRef]

56. U.S. Department of Agriculture. Taxon: *Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill; U.S. National Plant Germplasm System: Washington, DC, USA, 2021.

57. Wu, P.; Haseeb, M.; Zhang, R.; Kanga, L.H.B.; Legaspi, J.C. In vitro consumption patterns of pepper weevil, *Anthonomus eugenii* (Coleoptera: Curculionidae) on two commercial pepper cultivars in Florida. Appl. Entomol. Zool. 2019, 54, 473–479. [CrossRef]

58. Qureshi, J.; Kostyk, B.C. Insecticidal Control of Pepper Weevil on Jalapeño Pepper, Spring 2019. Arthropod Manag. Tests 2020, 45, tsaa027. [CrossRef]

59. Stommel, J.R.; Griesbach, R.J. Capsicum annuum L. ‘Black Pearl’. HortScience 2005, 40, 1571–1573. [CrossRef]

60. Yao, J.; Nair, M.G.; Chandra, A. Supercritical Carbon Dioxide Extraction of Scotch Bonnet (*Capsicum annuum*) and Quantification of Capsaicin and Dihydrocapsaicin. J. Agric. Food Chem. 1994, 42, 1303–1305. [CrossRef]

61. Marlin, E. Non-Pungent Ornamental Peppers. U.S. Patent 7,087,819 B2, 8 August 2006.

62. Marlin, E. Pepper line Hot Pops Yellow. U.S. Patent 9,307,712, 12 April 2016.

63. Addesso, K.M.; McAuslane, H.J. Pepper Weevil Attraction to Volatiles from Host and Nonhost Plants. Environ. Entomol. 2009, 38, 216–224. [CrossRef]

64. Idris, A.B.; Grafius, E. Effects ofWild and Cultivated Host Plants on Oviposition, Survival, and Development of Diamondback Moth (Lepidoptera: Plutellidae) and Its Parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). Environ. Entomol. 1996, 25, 825–833. [CrossRef]

65. Benrey, B.; Callejas, A.; Rios, L.; Oyama, K.; Denno, R.F. The Effects of Domestication of Brassica and Phaseolus on the Interaction between Phytophagous Insects and Parasitoids. Biol. Control 1998, 11, 130–140. [CrossRef]

66. Gols, R.; Bukovinszky, T.; Van Dam, N.M.; Dicke, M.; Bullock, J.M.; Harvey, J.A. Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. J. Chem. Ecol. 2008, 34, 132–143. [CrossRef] [PubMed]

67. Cuny, M.A.; Shlichta, G.J.; Benrey, B. The large seed size of domesticated lima beans mitigates intraspecific competition among seed beetle larvae. Front. Ecol. Evol. 2017, 5, 145. [CrossRef]

68. Berny-Mier y Teran, J.C.; Abdala-Roberts, L.; Durán-Yáñez, A.; Tut-Pech, F. Variation in insect pest and virus resistance among habanero peppers (*Capsicum chinense* Jacq.) in Yucatán, México. Agrociencia 2013, 47, 471–482.

69. Porter, P.; Lewis, B.E.; Scanlon, R.; Murrays, L. Pepper Weevil1 Infestation of Cv. Early Jalapeno Peppers of Different Size Classes. Southwest. Entomol. 2007, 32, 1–6. [CrossRef]

70. Silva, C.Q.; Jasmim, J.M.; Santos, J.O.; Bento, C.S.; Sudré, C.P.; Rodrigues, R. Phenotyping and selecting parents for ornamental purposes in chili pepper accessions. Hortic. Bras. 2015, 33, 66–73. [CrossRef]

71. Chaim, A.B.; Borovsky, Y.; Rao, G.; Gur, A.; Zamir, D.; Paran, I. Comparative QTL mapping of fruit size and shape in tomato and pepper. Isr. J. Plant Sci. 2006, 54, 191–203. [CrossRef]

72. Zygier, S.; Chaim, A.B.; Efrati, A.; Kaluzky, G.; Borovsky, Y.; Paran, I. QTLs mapping for fruit size and shape in chromosomes 2 and 4 in pepper and a comparison of the pepper QTL map with that of tomato. Theor. Appl. Genet. 2005, 111, 437–445. [CrossRef]

73. Riley, D.; Schuster, D.; Barfield, C. Sampling and dispersion of pepper weevil (Coleoptera: Curculionidae) adults. Environ. Entomol. 1992, 21, 1013–1021. [CrossRef]

74. Kim, M.; Oosterhuis, D.; Tugwell, N.; Bourland, F. Changes in the cotton boll wall in relation to boll weevil and bollworm feeding. Spe. Rep. Univ. Arkansas Agric. Exp. Stn. 1997, 183, 160–162.

75. Buczkowska, H.; Sałata, A.; Ro˙ zek, E. Diversity of the utility and biological value of fruits of some sweet pepper cultivars. Acta Sci. Pol. Hortorum Cultus 2014, 13, 49–62.

76. Gómez-Domínguez, N.S.; Refugio Lomeli-Flores, J.; Rodríguez-Leyva, E.; Valdez-Carrasco, J.M.; Torres-Ruiz, A. Ovipositor of *Catolaccus hunteri* Burks (Hymenoptera: Pteromalidae) and Implications for its Potential as a Biological Control Agent of Pepper Weevil. Southwest. Entomol. 2012, 37, 239–242. [CrossRef]

77. Hori, M.; Nakamura, H.; Fujii, Y.; Suzuki, Y.; Matsuda, K. Chemicals affecting the feeding preference of the Solanaceae-feeding lady beetle *Henosepilachna vigintioctomaculata* (Coleoptera: Coccinellidae). J. Appl. Entomol. 2011, 135, 121–131. [CrossRef]

78. Cowles, R.; Keller, J.; Miller, J. Pungent spices, ground red pepper, and synthetic capsaicin as onion fly ovipositional deterrents. J. Chem. Ecol. 1989, 15, 719–730. [CrossRef] [PubMed]

79. Weissenberg, M.; Klein, M.; Meisner, J.; Ascher, K. Larval growth inhibition of the spiny bollworm, *Earias insulana*, by some steroidal secondary plant compounds. Entomol. Exp. Appl. 1986, 42, 213–217. [CrossRef]

80. Ahn, S.-J.; Badenes-Pérez, F.R.; Heckel, D.G. A host-plant specialist, *Helicoverpa assulta*, is more tolerant to capsaicin from *Capsicum annuum* than other noctuid species. J. Insect Physiol. 2011, 57, 1212–1219. [CrossRef] [PubMed]

81. Ahn, S.J.; Badenes-Pérez, F.R.; Reichelt, M.; Svatoš, A.; Schneider, B.; Gershenzon, J.; Heckel, D.G. Metabolic detoxification of capsaicin by UDP-glycosyltransferase in three Helicoverpa species. Arch. Insect Biochem. Physiol. 2011, 78, 104–118. [CrossRef] [PubMed]

82. Baek, S.; Cho, K.; Song, Y.H.; Lee, J.-H. Sampling plans for estimating pepper fruit damage levels by Oriental tobacco budworm, *Helicoverpa assulta* (Guenee), in hot pepper fields. J. Asia Pac. Entomol. 2009, 12, 175–178. [CrossRef]

83. Fujiwake, H.; Suzuki, T.; Iwai, K. Capsaicinoid formation in the protoplast from the placenta of Capsicum fruits. Agric. Biol. Chem. 1982, 46, 2591–2592. [CrossRef]

84. Addesso, K.M.; McAuslane, H.J.; Alborn, H.T. Attraction of pepper weevil to volatiles from damaged pepper plants. Entomol. Exp. Appl. 2011, 138, 1–11. [CrossRef]

Supplementary material

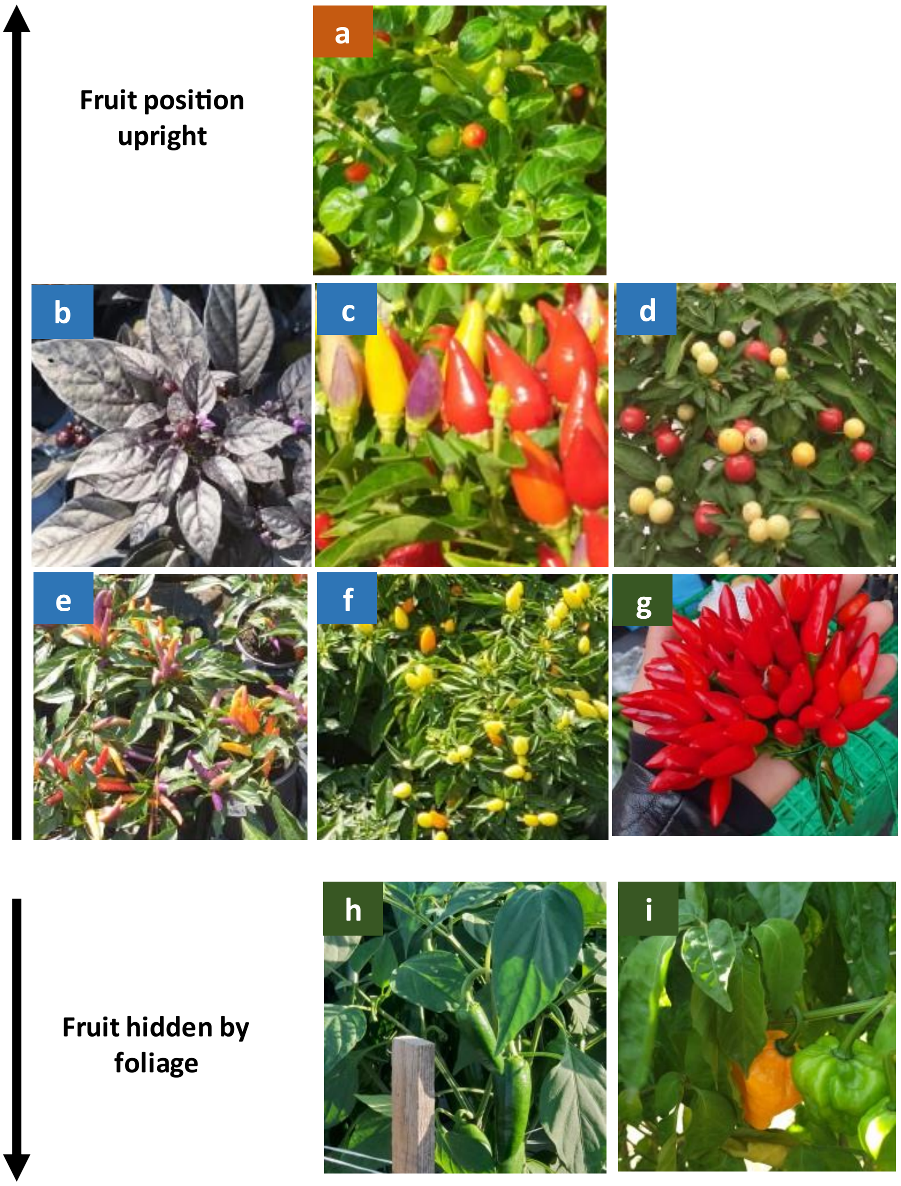
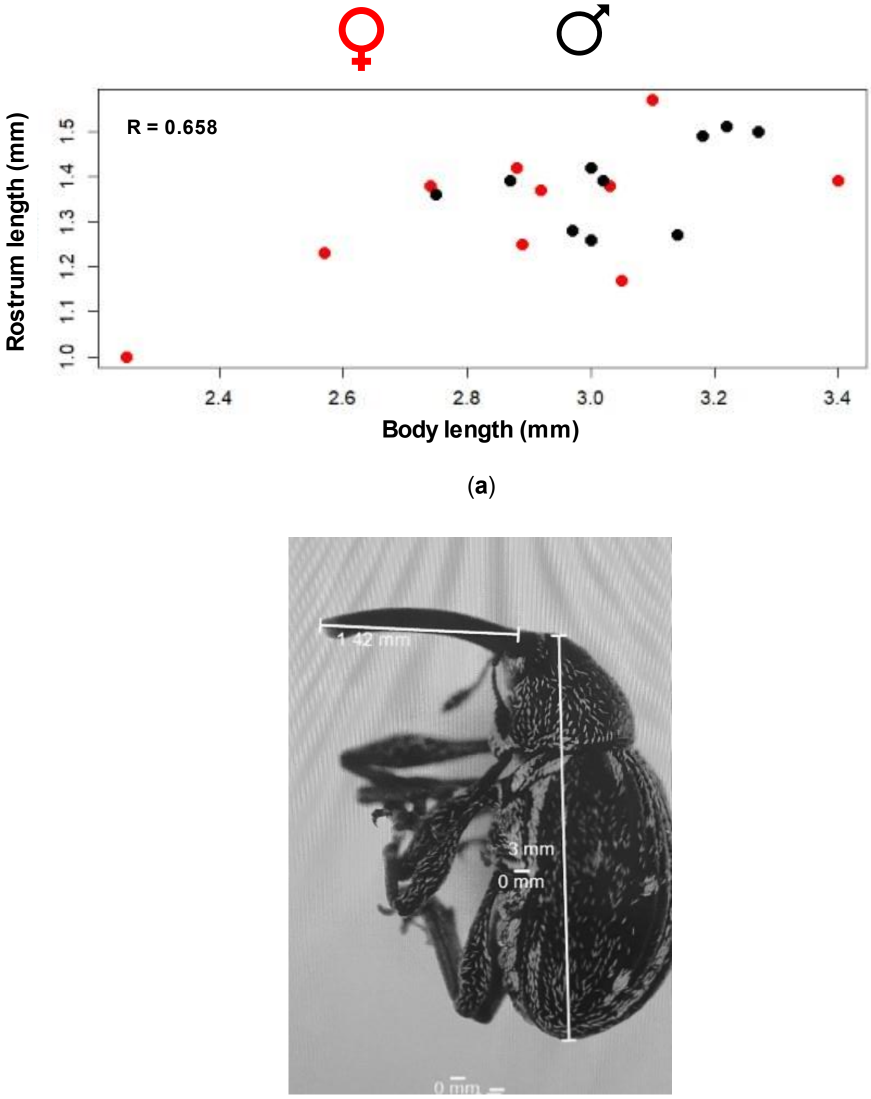


Fig. S1 Fruit position for wild (Brown), domesticated as ornamental (Blue), and domesticated for consumption (Green) peppers. All images © Y. Chabaane.



(a)

(b)

Fig. S2 (**a**) Correlation between body length (mm) and rostrum length (mm) using 10 males and 10 female weevils. Method used to test correlation = Pearson's product-moment correlation (R = **0.658). (b**) Measurement of body and rostrum lengths of a female weevil taken using a Leica DMS1000 microscope with build-in 5.0 MPixel CMOS Camera and LAS X Core software (ver. 3.0.13). © Y. Chabaane

5. General discussion

Plant domestication is defined as a plant selection process to suit human tastes and adapt to environmental conditions (Meyer and Purugganan 2013). As a result, there is raising evidence that several plant traits have been altered with direct and indirect consequences on multitrophic interactions (Chen et al. 2015b). However, for chili pepper, these consequences were poorly studied. Therefore, this thesis explored the altered traits in fruits, flowers, and leaves and investigated the effects of chili domestication on multitrophic interactions involving generalist and specialist herbivores, parasitoids, viruses, and birds.

5.1. Effects of altered capsaicinoids levels in fruits on tritrophic interactions

The process of crop domestication, selected for plant traits that favor human consumption, has often led to the loss of plant resistance against insect herbivores (Chen et al. 2015a; Fernandez et al. 2021; Whitehead et al. 2017). In this thesis, I found that chili domestication altered capsaicinoids levels in fruits, the main secondary metabolites of the *Capsicum* genus. I showed that nine wild Chiltepin accessions collected in Mexico (chapter 2) had an intermediate level of capsaicinoids between highly pungent varieties (Habanero in chapters 1 and 2 and Scotch Bonnet in chapter 3) and mild varieties (Cayenne in chapter 1, Patagonia in chapter 2, Jalapeño and ornamental varieties used for chapter 3). Indeed, chili pepper is a unique system consisting of a crop plant species, improved for both higher and lower concentrations of their secondary metabolites, so-called capsaicinoids (Scoville 1912). Currently, there is an increasing trend to select for highly pungent varieties (e.g., Carolina Reaper, the hottest chili variety) to suit the taste of “Chili Eating Contest Champions” around the world (Boddhula et al. 2018; Johnson 2016). However, the consequences of this trend on insects are poorly investigated.

In chapter 1, I found that the performance of the generalist caterpillar (*S. latifascia*) and its ectoparasitoid (*E. platyhypenae*) were significantly reduced when feeding on highly pungent fruits (Habanero) and a high-capsaicin diet. In addition, the beetle (*A. eugenii*) also prefers to feed on mild Jalapeño fruits than on spicy peppers (e.g., Scotch Bonnet, Wild Bird Eye, and the ornamental Black Pearl) despite being a specialist (chapter 3). Therefore, I expected that selecting for highly pungent varieties will enhance the protection of chili pepper against herbivorous insects. In turn, these latter were reported to use different strategies to cope with plant defenses, for instance, by 1) avoiding them (e.g., Feeding on plant tissue with lower defensive compounds (Shroff et al. 2008) or removing leaf hairs that prevent feeding (Medeiros and Moreira 2005)), (2) suppressing direct and indirect plant defenses using the secreted herbivore elicitors (Alba et al. 2011; De Lange et al. 2020), (3) or sequestering plant toxins and store them in their bodied making them unpalatable or toxic for their natural enemies (Opitz and Müller 2009). In my thesis, the analysis of *S. latifascia* haemolymph revealed both capsaicinoid compounds (capsaicin and dihydrocapsaicin) only when feeding on highly pungent Habanero variety (chapter 1). Despite being a generalist, this result suggests that *S. latifascia* could sequester capsaicinoids at high concentrations. These sequestered compounds might be responsible for the reduced parasitism rate of *E. platyhypenae*. Moreover, both larvae and adults of pepper weevils were observed feeding on the fruit's placenta of Scotch Bonnet variety (Chabaane, personal observation) where capsaicinoids are concentrated (Fujiwake et al. 1982). I expect this specialist beetle to handle chili spiciness and possibly sequester capsaicinoids since they feed on the placenta. This idea can be the subject of future study to unravel the mechanism used to cope with the toxicity of these secondary metabolites.

5.2. Effects of altered morphological fruit traits on insects

Plant domestication was also reported to alter morphological traits for many crops (e.g., maize, beans, and tomato) (Chen et al. 2015a). The increase of fruit or seed size is one of the most common morphological changes that arise from domestication to select for higher yield of several crops such as beans (*Phaseolus vulgaris*), maize (*Z. mays*), squash (*Cucurbita pepo*), tomato (*Lycopersicum esculentum*), and chili pepper (*Capsicum* spp) as compared to their wild ancestors (Cong et al. 2008; Cuny et al. 2017; Pickersgill 2016; Smith 1997; Studer et al. 2017). According to the plant vigor hypothesis, herbivores will prefer plant organs that grow faster and larger (Price 1991). In agreement with this hypothesis, Cuny et al. (2017) reported that the domestication of beans had been selected for larger seeds in cultivated plants, and as a consequence, the Mexican bean weevil *Zabrotes subfasciatus* lay more eggs on cultivated seeds but also larger adults emerged from these seeds. Our findings in chapter 3 support the above studies. Furthermore, the specialist pepper weevils fed and oviposit more on domesticated varieties for consumption with larger chili fruits than ornamental and wild chilies. However, the consequences of the fruit enlargement on the natural enemies of the pepper weevils should be further investigated. In their review, Chen et al. (2015a) reported a contracting result on the response of the third trophic level to fruit enlargement. On the one hand, the apple maggot fly (*Rhagoletis pomonella* (Walsh)), were less parasitized on the larger apple fruit than on small fruits from their native hawthorn trees (Feder 1995). On the other hand, Gomez and Zamora

(1994) found that weevils (*Ceutorhiynchus sp*) attacked larger fruits of *Hormathophylla spinosa* (L.) P than small fruits, but the parasitism rate was not dependant on the fruit size. In the case of pepper weevils, I expect their larvae developed inside the fruit would benefit from the large fruit size to escape parasitism by *Catolaccus hunteri,* one of the main parasitoids of these beetle. This idea remains to be tested.

Additionally, the selection for gigantism targeted the pericarp thickness for different crops such as bitter melon (*Momordica charantia* L.) and tomato (*Solanum.* spp) (Chaim et al. 2006; Marr et al. 2004). In cotton, the boll wall thickness was responsible for reduced damage on cotton plants caused by the boll weevil (*Anthonomus grandis grandis*, Boheman) (Kim et al. 1997). Chapter 3 showed that although chili domestication increased the pericarp thickness, this does not seem to influence fruit susceptibility to pepper weevil. A plausible explanation is that the maximum pericarp thickness recorded in our study (2.6 mm) was not enough to hamper the feeding and oviposition of these beetles. However, this measurement is doubled and may reach over 6 mm for some bell pepper cultivars (Buczkowska et al. 2014), thus explaining their resistance to pepper weevil (Riley et al. 1992).

Moreover, the domestication of chili pepper favored the loss of fruit dispersion by changing their position and removing their deciduous characteristic (Pickersgill 2016). In chapters 2 and 3, I reported that fruits of wild chilies had an upright position on the plant; however, most domesticated plants have their fruits hidden by foliage. Despite the vertical position of fruits from the Patagonia variety, birds interestingly removed only wilds fruits in the common garden experiment (chapter 2). This result suggests that the deciduous fruit characteristic of wild accessions enhanced their dispersion by birds.

5.3. Effects of altered flower and leaf traits on insect herbivores

Chili fruits, what else? The domestication syndrome for chili pepper mainly focused on changes in fruits, the main organ targeted by domestication (Bosland et al. 2012; Haak et al. 2012; Pickersgill 2016; Tewksbury and Nabhan 2001). However, altered traits in flowers and leaves of chili pepper were poorly investigated, and their consequences on multitrophic interaction remain unknown. Therefore, this thesis sheds light on these organs to explore the possible contrasting effect of domestication on different plant tissues. For lima beans, Shlichta et al. (2018) found that cyanogenic glycosides (CNGs) concentrations were altered in opposite directions both in seeds and leaves. Consequently, the beetle (*Zabrotes subfasciatus*) and the beet armyworm (*Spodoptera exigua)* preferred plant organs with low CNGs content, respectively, cultivated seeds and leaves of wild plants. In a recent study, cucurbitacins were detected in significant quantities in cotyledons and roots of Squash varieties (*Cucurbita* spp.) (Jaccard et al. 2021). In contrast, leaves have a high density of trichomes density but a minimal amount of cucurbitacins. This trade-off in plant tissue did not affect the performance of the leaf herbivore (*Spodoptera latifascia*) and the root beetle (*Diabrotica balteata*).

In chili pepper, leaves do not contain capsaicinoids (Diana La Forgia, master thesis). Therefore, I examined trichome density and phenolic compounds as potential defensive traits in leaves. Indeed, phenolics have been in the focus of many studies on plant defenses against leaf herbivores (Damestoy et al. 2019; Kumar et al. 2020; Pascual-Alvarado et al. 2008). They are antinutritive and toxic components against insects (Duffey and Stout 1996). They are also known for their antioxidant and antiviral activities (Rashad et al. 2020; Zhang et al. 2014). For example, Kofalvi and Nassuth (1995) found that infected wheat plants with mosaic streak virus had slightly more phenolic compounds in their leaves than non-infected plants. Our results from chapter 2 showed that there is a trade-off between physical and chemical defenses in leaves. As expected, the total trichome density in wild leaves was three times higher than on cultivated plants. This result may explain the greatest damage by chewing insects on chili varieties observed in the field. In turn, the phenolic content in leaves followed the opposite direction of trichome density. Therefore, a plausible explanation is that these compounds may play another role in the plant, protect chili varieties from UV stress than wild chilies that naturally grow in shaded areas (Kraft et al. 2013).

Additionally, chili pepper domestication enhanced the flower size and varied their color (chapter 3). Flower color did not appear to have any effect on weevil infestation and oviposition. However, the enlargement of the flower resulted in a more significant infestation and oviposition by the pepper weevil on domesticated varieties selected for consumption than on wild and ornamental peppers. Similarly, the domestication of sunflower (*Helianthus annuus* L.) enlarged their flower (Chen and Welter 2005). As a result, the larval abundance of the sunflower moth (*Homoeosoma electellum*) was increased, but they were four times less parasitized on agricultural sunflowers than on wild sunflowers (*H. annuus* var. *annuus*). Future work should investigate whether selection for gigantism may interfere with natural enemies of pepper weevil for chili pepper.

Moreover, results showed that pepper weevils fed but did not lay eggs on wild and ornamental flowers (chapter 3). It suggests that other floral traits (e.g., floral flavors responsible for the taste) might be necessary for selecting a plant host for their offspring. This possibility should be further studied. In addition, in this thesis, we focused on direct defenses for chili pepper. Further work should also consider chili plant volatiles from cultivated and wild plants and investigate the possible trade-off between direct and indirect defenses.

Taken together, the results from this thesis show that chili domestication has altered plant traits not only for fruits but also for leaves and flowers. Furthermore, these altered traits had both negative and positive impacts on multitrophic interactions. Thus, this thesis sheds light on a unique system of chili pepper, although worldwide consumed but neglected regarding the consequences of its domestication on insects and diseases. Further work is needed on this system to unravel other defensive traits possibly involved in these interactions. For a future perspective, our work could be implemented in a pest management program using mixed varieties to reduce insect and disease damage and breeding programs to select new resistant chili peppers.

5.4. Chiltepin and the improvement of domesticated chili

Plant breeding is crucial for developing and deploying new cultivars worldwide (Gepts and Hancock 2006). One of the breeding goals is to create resistant varieties against multiple pests (e.g., insects, pathogens, and viruses) (Chunthawodtiporn et al. 2019; Fraser 1992; Wink 1988). In this context, plant domestication plays an essential role in the first phase of a breeding program, creating genetic variability using the exotic germplasm (Ceccarelli 2015). Therefore, there is promising potential for using wild chilies (Chiltepin) to improve the nutritional quality of new chili varieties, yield, and efficiency for machine harvesting (Bosland 1996). Chiltepin has been substantially studied with regards to chili pepper evolution (Kraft et al. 2014; Perry and Flannery 2007; Pickersgill 1988; Tewksbury et al. 2008), their dispersion by birds (Egerer et al. 2018; Jordt and Julius 2002; Levey et al. 2006), and their tolerance to pathogens due to capsaicinoids (Adams et al. 2020; Gurung et al. 2015; Machnicki 2013). However, it has been ignored in research on insects and virus resistance. The research described in this thesis suggests that wild chilies had the greatest resistance compared to domesticated varieties, not only against the specialist insect pepper weevil (chapter 3) but also for generalist chewing insect and virus infestation on leaves (chapter 2). Therefore, breeding programs should not focus only on genes responsible for fruit resistance but also those that enhance leaf protection against biotic and abiotic stress.

Unfortunately, Chiltepin is at risk of becoming endangered in Mexico due to the overharvesting of the fruit, damage caused to plants by collectors and livestock, and the problem of tropical deforestation (Alma et al. 2020; Gentry 1942; Nabhan 1987). Therefore, a tremendous effort is undertaken to preserve its genetic resources using Mexican and American germplasm repositories, INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias) and USDA GRIN (United States Department of Agriculture’s Genetic Resources Information Network) (Kraft et al. 2013). Moreover, there are many other germplasm banks around the world dedicated to research and maintain the seed diversify of the *Capsicum* species, such as the Chile Pepper Institute (New Mexican State University), the Asian Vegetable Research and Development Center (ARVDC), and Banco de Germoplasma de Hortaliças in Brazil (BGH). However, in situ conservation of wild chilis is very limited and in dare straits (Meilleur and Hodgkin 2004; Tewksbury et al. 1999). Therefore, continuous efforts are still needed to maintain wild populations in their natural habitat to study the possible multitrophic interactions.

5.5. Outlook and future perspective

Taken together, the results of this thesis show that chili domestication has increased and decreased capsaicinoid in fruits. However, only at a high concentration of these secondary metabolites, capsaicin negatively affected insect herbivores and their ectoparasitoid. Therefore, the response of the plant to herbivory depends on the pungency level in fruits.

In leaves, we also showed that plant defenses were altered in the opposite direction, suggesting that domesticated varieties are not always less defended than their wild ancestors and that there is a possible trade-off between chemical and physical defenses in leaves. In this thesis, we focused on the constitutive defenses in chili pepper. However, the effects of domestication on chili-induced chemical defenses and whether there is a trade-off between induced and constitutive defenses remain unknown. In addition, we studied here the aboveground multitrophic interactions. However, the consequences of domestication on the belowground organisms associated with this crop are still unclear. Therefore, future research should examine the effects of chili domestication on the belowground community and the possible consequences on the aboveground system.

This work has important implications for sustainable agriculture that relies on enhanced plant resistance and reduced pesticide use. Moreover, the results of this thesis highlight the value of studying wild-domesticated systems. Finally, this study allows understanding the impact of short-term human-mediated selection pressures applied to our crop plants and the natural selection forces that have molded plant-insect interactions over evolutionary time.

References

Adams CA et al. (2020) Fungal Seed Pathogens of Wild Chili Peppers Possess Multiple Mechanisms To Tolerate Capsaicinoids Applied and Environmental Microbiology 86:e01697-01619 doi:https://doi.org/10.1128/AEM.01697-19

Alba JM, Glas JJ, Schimmel BC, Kant MR (2011) Avoidance and suppression of plant defenses by herbivores and pathogens Journal of Plant Interactions 6:221-227 doi:https://doi.org/10.1080/17429145.2010.551670

Alma AV-F et al. (2020) Pytochemical profile and antioxidant activity of chiltepin chili (*Capsicum annuum* var. *glabriusculum*), Sonora, Mexico Journal of Food Bioactives 11 doi:https://doi.org/10.31665/JFB.2020.11237

Boddhula SK, Boddhula S, Gunasekaran K, Bischof E (2018) An unusual cause of thunderclap headache after eating the hottest pepper in the world–"The Carolina Reaper" Case Reports 2018:bcr-2017-224085 doi:http://dx.doi.org/10.1136/bcr-2017-224085

Bosland PW (1996) Capsicums: Innovative uses of an ancient crop Progress in new crops ASHS Press, Arlington, VA:479-487

Bosland PW, Votava EJ, Votava EM (2012) Peppers: vegetable and spice capsicums vol 22. Cabi. doi:10.1080/01140671.2012.745161

Buczkowska H, Sałata A, Rożek E (2014) Diversity of the utility and biological value of fruits of some sweet pepper cultivars Acta Sci Pol, Hortorum Cultus 13:49-62

Ceccarelli S (2015) Efficiency of plant breeding Crop Science 55:87-97 doi:https://doi.org/10.2135/cropsci2014.02.0158

Chaim AB, Borovsky Y, Rao G, Gur A, Zamir D, Paran I (2006) Comparative QTL mapping of fruit size and shape in tomato and pepper Israel Journal of Plant Sciences 54:191-203 doi:10.1560/IJPS_54_3_191

Chen YH, Gols R, Benrey B (2015a) Crop domestication and its impact on naturally selected trophic interactions. Annual Review of Entomology 60:35-58. doi:https://doi.org/10.1146/annurev-ento-010814-020601

Chen YH, Gols R, Stratton CA, Brevik KA, Benrey B (2015b) Complex tritrophic interactions in response to crop domestication: predictions from the wild. Entomologia Experimentalis et Applicata 157:40-59. doi:https://doi.org/10.1111/eea.12344

Chen YH, Welter SC (2005) Crop domestication disrupts a native tritrophic interaction associated with the sunflower, *Helianthus annuus* (Asterales: Asteraceae) Ecological Entomology 30:673-683 doi:https://doi.org/10.1111/j.0307-6946.2005.00737.x

Chunthawodtiporn J, Hill T, Stoffel K, Van Deynze A (2019) Genetic Analysis of Resistance to Multiple Isolates of *Phytophthora capsici* and Linkage to Horticultural Traits in Bell Pepper HortScience 54:1143-1148

Cong B, Barrero LS, Tanksley SD (2008) Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication Nature Genetics 40:800-804 doi:https://doi.org/10.1038/ng.144

Cuny MA, Shlichta GJ, Benrey B (2017) The large seed size of domesticated lima beans mitigates intraspecific competition among seed beetle larvae Frontiers in Ecology and Evolution 5:145 doi:https://doi.org/10.3389/fevo.2017.00145

Damestoy T, Brachi B, Moreira X, Jactel H, Plomion C, Castagneyrol B (2019) Oak genotype and phenolic compounds differently affect the performance of two insect herbivores with contrasting diet breadth Tree Physiology 39:615-627 doi:10.1093/treephys/tpy149

De Lange ES et al. (2020) *Spodoptera frugiperda* Caterpillars Suppress Herbivore-Induced Volatile Emissions in Maize Journal of Chemical Ecology 46:344-360 doi:https://doi.org/10.1007/s10886-020-01153-x

Duffey SS, Stout MJ (1996) Antinutritive and toxic components of plant defense against insects Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America 32:3-37

Egerer MH, Fricke EC, Rogers HS (2018) Seed dispersal as an ecosystem service: frugivore loss leads to decline of a socially valued plant, *Capsicum frutescens* Ecological applications 28:655-667 doi:https://doi.org/10.1002/eap.1667

Feder JL (1995) The effects of parasitoids on sympatric host races of *Rhagoletis pomonella* (Diptera: Tephritidae) Ecology 76:801-813 doi:https://doi.org/10.2307/1939346

Fernandez AR, Sáez A, Quintero C, Gleiser G, Aizen MA (2021) Intentional and unintentional selection during plant domestication: herbivore damage, plant defensive traits and nutritional quality of fruit and seed crops New Phytologist doi:https://doi.org/10.1111/nph.17452

Fraser RSS (1992) The genetics of plant-virus interactions: implications for plant breeding Euphytica 63:175-185 doi:https://doi.org/10.1007/BF00023922

Fujiwake H, Suzuki T, Iwai K (1982) Capsaicinoid formation in the protoplast from the placenta of Capsicum fruits. Agricultural and Biological Chemistry 46:2591-2592. doi:https://doi.org/10.1080/00021369.1982.10865477

Gentry HS (1942) Rio Mayo plants. Carnegie Institution,Washington, DC No. 257.

Gepts P, Hancock J (2006) The future of plant breeding crop Science 46:1630-1634 doi:https://doi.org/10.2135/cropsci2005-12-0497op

Gomez JM, Zamora R (1994) Top-down effects in a tritrophic system: parasitoids enhance plant fitness Ecology 75:1023-1030 doi:https://doi.org/10.2307/1939426

Gurung S, Short DP, Hu X, Sandoya GV, Hayes RJ, Subbarao KV (2015) Screening of wild and cultivated Capsicum germplasm reveals new sources of Verticillium wilt resistance Plant disease 99:1404-1409 doi:https://doi.org/10.1094/PDIS-01-15-0113-RE

Haak DC, McGinnis LA, Levey DJ, Tewksbury JJ (2012) Why are not all chilies hot? A trade-off limits pungency. Proceedings of the Royal Society B: Biological Sciences 279:2012-2017. doi:https://doi.org/10.1098/rspb.2011.2091

Johnson AR (2016) The Art of Competitive Eating Gastronomica 16:111-114 doi:https://doi.org/10.1525/gfc.2016.16.3.111

Jordt S-E, Julius D (2002) Molecular basis for species-specific sensitivity to “hot” chili peppers. Cell 108:421-430. doi:https://doi.org/10.1016/S0092-8674(02)00637-2

Kim M, Oosterhuis D, Tugwell N, Bourland F (1997) Changes in the cotton boll wall in relation to boll weevil and bollworm feeding special reports-university of arkansas agricultural experiment station 183:160-162

Kofalvi SA, Nassuth A (1995) Influence of wheat streak mosaic virus infection on phenylpropanoid metabolism and the accumulation of phenolics and lignin in wheat Physiological and Molecular Plant Pathology 47:365-377 doi:https://doi.org/10.1006/pmpp.1995.1065

Kraft KH et al. (2014) Multiple lines of evidence for the origin of domesticated chili pepper, Capsicum annuum, in Mexico Proceedings of the National Academy of Sciences 111:6165-6170

Kraft KH, de Jesús Luna-Ruíz J, Gepts P (2013) A new collection of wild populations of Capsicum in Mexico and the southern United States Genetic Resources and Crop Evolution 60:225-232 doi:https://doi.org/10.1007/s10722-012-9827-5

Kumar S, Abedin MM, Singh AK, Das S (2020) Role of phenolic compounds in plant-defensive mechanisms. In: Plant Phenolics in Sustainable Agriculture. Springer, pp 517-532. doi:https://doi.org/10.1007/978-981-15-4890-1_22

Levey DJ, Tewksbury JJ, Cipollini ML, Carlo TA (2006) A field test of the directed deterrence hypothesis in two species of wild chili Oecologia 150:61-68 doi:https://doi.org/10.1007/s00442-006-0496-y

Machnicki NJ (2013) How the chili got its spice: ecological and evolutionary interactions between fungal fruit pathogens and wild chilies.

Marr KL, Mei XY, Bhattarai NK (2004) Allozyme, morphological and nutritional analysis bearing on the domestication of *Momordica charantia* L.(Cucurbitaceae) Economic Botany 58:435-455 doi:https://doi.org/10.1663/0013-0001(2004)058[0435:AMANAB]2.0.CO;2

Medeiros L, Moreira GR (2005) Larval feeding behavior of *Gratiana spadicea* (Klug)(Coleoptera: Chrysomelidae: Cassidinae) on its host plant, *Solanum sisymbriifolium* Lamarck (Solanaceae): Interaction with trichomes The Coleopterists Bulletin 59:339-350 doi:https://doi.org/10.1649/778.1

Meilleur BA, Hodgkin T (2004) In situ conservation of crop wild relatives: status and trends Biodiversity & Conservation 13:663-684 doi:https://doi.org/10.1023/B:BIOC.0000011719.03230.17

Meyer RS, Purugganan MD (2013) Evolution of crop species: genetics of domestication and diversification Nature reviews genetics 14:840-852 doi:https://doi.org/10.1038/nrg3605

Nabhan G (1987) Nurse plant ecology of threatened desert plants

Opitz SE, Müller C (2009) Plant chemistry and insect sequestration. Chemoecology 19:117-154. doi:https://doi.org/10.1007/s00049-009-0018-6

Pascual-Alvarado E, Cuevas-Reyes P, Quesada M, Oyama K (2008) Interactions between galling insects and leaf-feeding insects: the role of plant phenolic compounds and their possible interference with herbivores Journal of Tropical Ecology 24:329-336 doi:https://doi.org/10.1017/S0266467408005038

Perry L, Flannery KV (2007) Precolumbian use of chili peppers in the Valley of Oaxaca, Mexico Proceedings of the National Academy of Sciences 104:11905-11909 doi:https://doi.org/10.1073/pnas.0704936104

Pickersgill B (1988) The genus Capsicum: a multidisciplinay approach to the taxonomy of cultivated and wild plants.

Pickersgill B (2016) Chile Peppers (*Capsicum* spp.). In: Lira R, Casas A, Blancas J (eds) Ethnobotany of Mexico: Interactions of People and Plants in Mesoamerica. Springer New York, New York, NY, pp 417-437. doi:https://doi.org/10.1007/978-1-4614-6669-7_17

Price PW (1991) The plant vigor hypothesis and herbivore attack Oikos:244-251

Rashad Y, Aseel D, Hammad S (2020) Phenolic Compounds Against Fungal and Viral Plant Diseases. In: Plant Phenolics in Sustainable Agriculture. Springer, pp 201-219. doi:https://doi.org/10.1007/978-981-15-4890-1_9

Riley D, Schuster D, Barfield C (1992) Sampling and dispersion of pepper weevil (Coleoptera: Curculionidae) adults Environmental entomology 21:1013-1021 doi:https://doi.org/10.1093/ee/21.5.1013

Scoville WL (1912) Note on capsicums. Journal of the American Pharmaceutical Association 1:453-454. doi:https://doi.org/10.1002/jps.3080010520

Shlichta JG, Cuny MA, Hernandez-Cumplido J, Traine J, Benrey B (2018) Contrasting consequences of plant domestication for the chemical defenses of leaves and seeds in lima bean plants. Basic and Applied Ecology 31:10-20. doi:https://doi.org/10.1016/j.baae.2018.05.012

Shroff R, Vergara F, Muck A, Svatoš A, Gershenzon J (2008) Nonuniform distribution of glucosinolates in Arabidopsis thaliana leaves has important consequences for plant defense Proceedings of the National Academy of Sciences 105:6196-6201 doi:https://doi.org/10.1073/pnas.0711730105

Smith BD (1997) The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago Science 276:932-934 doi:10.1126/science.276.5314.932

Studer AJ, Wang H, Doebley JF (2017) Selection during maize domestication targeted a gene network controlling plant and inflorescence architecture Genetics 207:755-765 doi:https://doi.org/10.1534/genetics.117.300071

Tewksbury JJ, Nabhan GP (2001) Directed deterrence by capsaicin in chillies. Nature 412:403-404. doi:https://doi.org/10.1038/35086653

Tewksbury JJ, Nabhan GP, Norman D, Suzán H, Tuxill J, Donovan J (1999) In situ conservation of wild chiles and their biotic associates Conservation Biology 13:98-107 doi:https://doi.org/10.1046/j.1523-1739.1999.97399.x

Tewksbury JJ, Reagan KM, Machnicki NJ, Carlo TA, Haak DC, Peñaloza ALC, Levey DJ (2008) Evolutionary ecology of pungency in wild chilies. Proceedings of the National Academy of Sciences 105:11808-11811. doi:https://doi.org/10.1073/pnas.0802691105

Whitehead SR, Turcotte MM, Poveda K (2017) Domestication impacts on plant–herbivore interactions: a meta-analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 372:20160034. doi:https://doi.org/10.1098/rstb.2016.0034

Wink M (1988) Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores Theoretical and applied genetics 75:225-233

Zhang X-L et al. (2014) Phenolic compounds from *Origanum vulgare* and their antioxidant and antiviral activities Food Chemistry 152:300-306 doi:https://doi.org/10.1016/j.foodchem.2013.11.153.

6. Acknowledgements

It is getting very spicy…I think it is time to end this Ph.D.

This work was done thanks to international teams:

Switzerland

Betty Benrey: "*I like chili, but not enough to discuss it with someone from Mexico*", I think we are the perfect match for this project. We both love chilies and grow up eating them since we are a child. As high as Carolina Reaper, I would like to express my sincere gratitude to you for giving me this opportunity to carry out my research in your laboratory. Thanks a lot for your guidance and tremendous support during these four years of my Ph.D. I have learned much from you. You are an excellent supervisor and a wonderful human being! It was a great pleasure working with you, Betty.

Ted Turlings: Thanks for supporting my application for the FCS Scholarship. I remember you told me after my master, "*You have to be crazy to do a Ph.D.,*" and here I am, getting crazy with chili pepper!!! Thank you also for being with us for my private defense!

Tim Haye: Danke schön! I was so honored to have you in my thesis committee. Thank you for your comments, advice and attending my public presentation as well.

Carla Arce: It was such a great pleasure to know you and sharing the same office. You always tried to help me as much as possible. I am very grateful for your help and support. *Míra*, thank you so much for your advice, guidance, and open heart. *Pero,* please no more 5 CHF charges (joke!!).

Pilar Junier: Thanks for the comments on my thesis!

Gaétan Glauser: Thank you for your help with the chemical analysis.

Pamela Bruno: *Almost there Pam, Hopp!!* Many thanks for the nice moments we shared during this Ph.D. It was lovely having you around!

Ilham Sbaiti and Diane Laplanche: I miss so much our lunch breaks by the lakeside !! Thank you so much for your support until the end of my Ph.D.

Hanaa Sarhan: You open your heart, your house, and even your office for me! I went through many storms during these four years, and you have always been here to support me. Endless thank you, Hanouuu. Having you as a friend is one of the beautiful outcomes of this Ph.D.

Evol team: Big thanks, especially to Carlos, Charlyne, Lucas, Elsa, Fanny, for their help in the fieldwork and here in Neuchâtel as well.

Marine: Thanks for helping me to bring the plants from the botanical garden and the friendly chats!

My friend Elvira Delange: Thank you so much for all your encouragement and support since my master. Your friendship is one of the best gifts that Switzerland gave me! I hope to meet soon again.

Evol-Farce team: For me, it was like going back to my second family. It is always lovely to be part of this big team. Keep going!

Botanical garden of Neuchâtel: I would like to express my thanks to the whole team and specifically to Nicolas Ruch and Elisabeth Baguet Oppliger for taking care of my chili plants.

This Ph.D. was funded by:
The Federal Commission for Scholarships for Foreign Students (FCS): I was honored with the Swiss Excellence Scholarship to conduct my Ph.D. I would not have the opportunity to pursue my research without this grant. I am very grateful to the FCS committee for this opportunity.
The University of Neuchâtel, Institute of Biology: I am very grateful for accepting my Ph.D. application, giving me the position of an assistant doctorant that allow me to finish my research and gave me the chance to teach both bachelor and master students.
The Egalité des chances grant to conduct my fieldwork in Mexico for two successive years, 2019 and 2020. Huge thank you for your generosity and trust!
The Interuniversity Doctoral Program in Organismal Biology (UNINE) allows me to conduct my research in Tallahassee, Florida. Thank you so much!

Mexico

Raul Gonzales: Thank you so much for all the work with us in the field and for taking care of my plants before and after I leave. I want to say also I really appreciate your support whenever I felt exhausted during the fieldwork. Big thanks, Raul!
Team of Universidad del Mar: Special thanks go to Alfredo, Rufi, Meriam, Betty to make our fieldwork possible and allow me to access their laboratory facilities as well.
Victor: *The right man, in the right place*. Muchas Gracias for helping me sampling wild chilies. Without your help, I will not be able to access many locations.
Puerto Escondido: *La Bonna Fortuna*, very nice memories with lovely people there: Muchas Gracias to Liz, Lozodelli team, Clara, Nico, Ohlii, William, and locals that help me to sample wild chilies. I was impressed by their generosity and willingness to share their stories about chilies.

United States of America

Muhammed Hasseed: I am very grateful for the invitation and for hosting my research on the pepper weevil in your laboratory at Florida A&M University (FAMU).

Donna Arnold, Briana, and Warell: My Jamaican friends, thanks a lot for hosting me at your house, for your kindness and your support during my journey in Tallahassee. It was great knowing you.

Jermaine, Alberta, Halimah, and Elan: Many thanks for your help collecting insects and fruits in the field, taking care of plants, and assisting with the bioassays.

My family in Tunisia

My parents Amina and Monji: Since I was a child, you tried your best to give me the best opportunities for my studies, even though it was sometimes challenging. I am incredibly grateful for all your encouragement, and I am very honored to have you, my parents! *Enhibkom barcha w Rabii ey fadhelkom laya ya ghalin!*

Oumi Meriem and Baba Azizi (Grandparents): Huge part of my personality is thanks to the values you teach me. I will never forget your advice “Drop unnecessary subjects from your life and look forward.” Words can not describe how much I am thankful and privileged to grow up with you!

My little brother Rabii: I know you are not that little, but for me, you will always be like that in my eyes! Thanks for your encouragement and supportive words whenever I felt exhausted.

The rest of my family: Many thanks, especially to my aunt Mounira and my uncles Fathi, Lassaad, and Khaled, for their support and lovely wishes.

To Ameni and Mohamed Mkaouer: Thank you my dear friends and family for all your support and advices. We will always stay close despite distance!

My family in Switzerland

My brother Rafat: You always encourage me to be the best I can. Enormous thanks for your encouragement. I want to say thank you for the nice company and support, especially during the Corona lockdown, also to my sister-in-law Natacha and my lovely nices Sara and Lilia.

My uncle Issam and my aunt Stephanie: Thanks to you, I grow up with the dream to study here in Switzerland. Thanks for all your support to make a dream come true! Huge thanks also to my cousins Ines, Farah, and Bilel for their encouragement and the lovely moments we spend in Lausanne that I needed to recharge my batteries.

My husband in Canada

To Souhail: I am counting days to see you and to be finally living together. Despite the distance and the time difference, you tried your best to be with me and support me during challenging periods! Your patience and smile gave me the strength to continue until the end. Eyaychik Habibi = Thank you ! Finally, after one year, eight months, and 14 days, we will hopefully meet!

7. Curriculum vitae

Yosra Chabaane
yosra.chabaane@bluewin.ch

Education

2017 - Aug 2021 **Ph.D.,** Biology, University of Neuchâtel (UNINE) - SWITZERLAND
Thesis: Consequences of the domestication of chili pepper on multitrophic interactions (plant, herbivores, natural enemies, and virus)
Supervisor: Prof. Betty Benrey - Laboratory of Evolutionary Entomology.

2011 - 2013 **Master in Plant Physiology and Ecology,** the University of Neuchâtel - SWITZERLAND
Thesis: Impact of exotic insect herbivores on native tritrophic interactions
Supervisor: Prof. Ted Turlings - Laboratory of Fundamental and applied research in chemical ecology.

2005 - 2010 **Engineer in Agronomy,** Institut National Agronomique de Tunis - TUNISIA
Thesis: Evaluation of different irrigation monitoring tools used for potato crop
Supervisor: Dr. Ali Sahli – Department of Agronomy & Plant Biotechnology.

Honours

2017 - 2021 **Swiss Government Excellence Scholarship**

Research interests

- Applied Entomology
- Plant Domestication
- Invasive insects
- Chemical Ecology of plant-insect interactions

Additional research experience

Sep to Oct 2019 **Visiting Doctoral Student at Florida Agricultural and Mechanical University, Florida - USA**

- Evaluating the feeding and oviposition of pepper weevil (*Anthonomus eugenii,* Cano) on wild and domesticated chili varieties.
- Conducting field trapping and rearing of this pest.

Nov to Feb 2019 & Dec to Feb 2020
Fieldwork at Universidad del Mar of Puerto Escondido - Mexico

- Studying the insect community associated with chili pepper in their domestication area using a common garden set up where domesticated and wild plants grow in an open field.
- Conducting bioassays to investigate the impact of chili domestication on local herbivores and their natural enemies.
- Sampling nine populations of wild chilies in the region of Puerto Escondido.

Apr 2013 - Jun 2013
Research assistant in the section on biological weed control at CABI, Delémont - Switzerland & Dresden - Germany

- Working on projects for *Pilosella spp.* (hawkweeds), *Fallopia Japonica* (Japaneese Knotweed) and *Convolvulus arvensis* (field bindweed).
- Evaluation of bioassays to determine the host range of potential insect biological control agents on projects mentioned above.
- Maintenance of insect rearing colonies under quarantine conditions.
- Helping with plant, insect, and fungal pathogen collections in Eastern Germany and Switzerland.

Apr 2012 - Apr 2013
Scientific collaborator at UNINE - Switzerland

- Conducting various behavioral experiments using a six-arm olfactometer.
- Collecting and analysing plant volatiles (with GC-MS).
- Using the electroantennogram technique for the parasitoid wasp *Cotesia glomerata.*
- Insect rearing includes exotic species restricted to quarantine conditions (over six different herbivores and parasitoids).

Teaching experience

Sep 2020 - present
Co-supervisor a master student, UNINE - Switzerland

- Thesis project: Behaviour of the southern green stink bug (*Nezara viridula*) as a response to chili pepper domestication.

Spring semesters 2019, 2020 and 2021
APP course (Apprentissage par problème), UNINE - Switzerland

- Supervising 3rd-year bachelor students to conduct research projects on plant domestication and multitrophic interactions.
- Helping students conceptualize their projects, conducting behaviour experiments, chemical and data analysis to answer their research questions.

Spring semesters 2012 and 2018
Teaching assistant for the practical course of entomology, UNINE - Switzerland

- Teaching the internal anatomy, external morphology, classification of major insect genus, and techniques to collect them in the field.

Non-academic professional experience

Apr 2014 - Jul 2015 **Intern then a consultant at FAO (Food and Agriculture Organisation of the United Nations), HQ Rome - Italy**

- Coordinate with experts and country members worldwide to manage the standard-setting process, and facilitate the production of diagnostic protocols related to regulated pests.
- Assist in the organization and attend different meetings :
- **In Rome (FAO, HQ)**: Three Standard Committee meetings and one CPM (Commission on Phytosanitary Measures) meeting.
- **In Shanghai, China**: The Technical Panel on Diagnostic Protocol meeting (June 2015).
- Draft meeting reports and assist with the development of technical paper discussions.

Oct 2016 - Apr 2017 **Product Manager at the Startup nextProtein, Nabeul - Tunisia**

- Conducting agronomic tests to evaluate an organic fertilizer extracted from the Black Soldier Fly (BSF).
- Establish the commercial feasibility study for the Tunisian and European markets.

Publications

Chabaane, Y., Laplanche, D., Turlings, T.C.J. & Desurmont, G.A. (**2015**) Impact of exotic insect herbivores on native tritrophic interactions: a case study of the African cotton leafworm, *Spodoptera littoralis* and insects associated with the field mustard *Brassica rapa*. **Journal of Ecology**, 103, 109–117.

Chabaane, Y., Marques Arce, C., Glauser, G. et al. Altered capsaicin levels in domesticated chili pepper varieties affect the interaction between a generalist herbivore and its ectoparasitoid. **J Pest Sci (2021)**

Chabaane Y, Haseeb M, Benrey B **(2021)** Domestication of Chili Pepper Has Altered Fruit Traits Affecting the Oviposition and Feeding Behavior of the Pepper Weevil **Insects** 12:630

Conferences attended & organized

2018	The 24th International Pepper Conference, Florida – USA.
2018	Biology 18 Meeting, Neuchâtel - Switzerland.
2014	International Conference on Nutrition (ICN2) organized by FAO et WHO (World Health Organisation), Rome - Italy.
2013	Final Conference for the National Centres of Competence in Research, Neuchâtel - Switzerland.

Additional certificate

June 2020 **Innosuisse Start-up Training on Business Concept** (A Swiss Federal program for startup founders)
A 14-week training program of different skills needed to develop a business project (e.g., project management, entrepreneurial thinking, financing, intellectual properties, presentation skills).

Langues

Fluent in French and English
Arabic (Mother's tongue)

Awarded grants

- Grant of « *Egalité des chances* » awarded two successive years, 2018 and 2019, to conduct fieldwork in Mexico.
- Grant for fieldwork awarded by the Federal Commission for Scholarships for Foreign Students.
- Grant of the Interuniversity Doctoral Program in Organismal Biology.