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Agroecosystem resilience to an invasive insect species that could expand its geographical range in response to global climate change☆

Martín Aluja[a,*], Andrea Birke[a], Maria Ceymann[b], Larissa Guillén[a], Eva Arrigoni[b], Daniel Baumgartner[b], Carlos Pascacio-Villafán[a], Jörg Samietz[b,1]

[a] Instituto de Ecología, A.C., Apartado Postal 63, 91000 Xalapa, Veracruz, Mexico
[b] Agroscope Changins-Wädenswil ACW, Schloss 1, CH-8820 Wädenswil, Switzerland

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ABSTRACT

We examined the attack of a potentially invasive tropical insect on a non-optimal temperate zone host and tested the hypothesis that variation in plant secondary metabolites and/or locally-grown host plant cultivars could shape agroecosystem resilience in a region undergoing climatic change. We studied the phytophagous fruit fly *Anastrepha ludens* (Diptera: Tephritidae) and 18 apple cultivars most of which vary significantly in total content of phenolic compounds. High content of phenolic compounds significantly increased egg or larval mortality whereas cultivars exhibiting low content were severely infested. Intermediate concentrations resulted in pupal malformation and delayed immature development. These results provide a valuable insight into biotic factors that contribute to environmental resilience to an invasive species that could expand its geographical range in response to global climate change. They also highlight the importance of protecting ancestral or locally-grown apple cultivars as sources of genes for breeding programs directed at restoring the ability of crops to defend themselves against emerging pests or to cope with changing environmental conditions.

1. Introduction

Understanding the factors that modulate ecosystem function constitutes one of the central interests of ecology. This understanding acquires special relevance in the face of novel environmental scenarios produced by human activity (Loreau et al., 2001; Butchart et al., 2010). Global warming-linked variation in the distribution and abundance of living organisms, ecological function and the magnitude and direction of their interactions has attracted particular interest (e.g., Menéndez, 2007; Deutsch et al., 2008; Robinet and Roques, 2010; Traill et al., 2010). Global climate change favors the introduction and establishment of species to new environments with a potentially devastating impact on agriculture and ecosystems (Dukes and Mooney, 1999). An increase in global temperature will likely create suitable conditions for the displacement of insects from tropical areas to temperate regions (Dukes and Mooney, 1999; Bidart-Bouzat et al., 2005; Parmesan, 2006; Bidart-Bouzat and Imeh-Nathaniel, 2008; Aluja et al., 2011). This will be easier for phytophagous insects that can adapt to new environments where suitable host plants are present (Bale et al., 2002; Birke et al., 2013), as well as for polyphagous insects that have the ability to exploit new hosts (Harrington et al., 2001; Logan and Powell, 2001). For example, global warming appears to have aided the Walnut Husk Fly, *Rhagoletis completa* Cresson (a pest of walnuts native to North America), to cross the Swiss Alps and establish populations in most of Switzerland and several other European countries (Aluja et al., 2011).

Climate change-driven pest invasion will be modulated by ecosystem resilience, that is, the capability of a system to tolerate a certain degree of change while maintaining functionality (Gunderson, 2000; Mijatović et al., 2013). Resilient natural or agricultural ecosystems are expected to remain functional even if challenged by severe disturbance (Lin, 2011). However, the resilience of agricultural systems is often reduced as a consequence of functional diversity loss caused by intensive agriculture or by reduced resistance in cultivated plants due to intensive breeding (Ekström and Ekbom, 2011). The former requires a complete redesign of productive systems, whereas the latter can be addressed by reincorporating lost intrinsic resilience, an issue that will likely be the focus of research in the years to come as functional agroecosystems come under increasing pressure from climatic change and human population growth.

Plant resistance to insect attack is achieved principally by physical and chemical defense mechanisms (particularly plant secondary

☆ We dedicate this paper to the memory of the late Jörg Samietz, who was integral part of this research, but unfortunately died suddenly while doing what he most enjoyed: conducting research.

* Corresponding author.
E-mail address: martin.aluja@inecol.mx (M. Aluja).
[1] Deceased.

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metabolites) that in the case of commercial cultivars have been lost or diminished during domestication processes focused on palatability to humans or yield (Aluja and Mangan, 2008; Kellerhals, 2009) or are varying due to global-scale environmental change (Iason et al., 2012). During these processes, genetic information responsible for encoding insect and pathogen resistance was either diluted (Rodriguez-Saona et al., 2011) or eliminated (Obeso, 2002). Specifically, the concentration of secondary metabolites, such as phenolic compounds (e.g., phenolic acids, flavonoids) that play an active role in plant defense has been reduced in plants under selection for agriculture (Treutter, 2006, 2010). This is relevant in two contexts. First, it presents the opportunity to test hypotheses related to ecological resilience under future global change scenarios as phenolic compounds are predicted, in a global warming scenario, to be metabolized at higher rates due to increased CO_2 and O_3 concentrations (Bidart-Bouzat et al., 2005; Himanen et al., 2008; Côté and Darling, 2010), and second, it is related to practical aspects associated with managing the inherent risk of invasion both in natural and agricultural ecosystems (Ward and Masters, 2007).

Knowledge on the effect of plant secondary metabolites on herbivorous insects is crucial to predict host plant resistance (Bennett and Wallsgrove, 1994), an indicator of environmental resilience in the context of this study. Here, we tested the capacity of locally-grown and commercial apple (*Malus* × *domestica* Borkh.) cultivars (genotypes), that differ in their total content of phenolic compounds, to resist the attack of a potentially invasive polyphagous insect pest, the Mexican fruit fly, *Anastrepha ludens* [Loew] (Diptera: Tephritidae). Studies were performed in Switzerland and Mexico. By 2050, mean winter temperatures in Switzerland are expected to increase by about 1.8 °C and summer temperatures by about 2.7 °C compared to 1990 (OcCC, 2007). In Mexico, apple growing areas are presently located less than 50 km away from where *A. ludens* occurs naturally, and mean temperatures are expected to increase by 1.1 to 3.0 °C before 2060 (McSweeney et al., 2008, 2010). In testing the prediction that high content of phenolic compounds in fruit would negatively influence the ability of this fly to invade apple agroecosystems in temperate regions, or mountainous areas, we show that locally-grown apple cultivars (i.e., non-commercial apple cultivars grown by local farmers over many years/centuries in their orchards), are better defended than commercial cultivars (i.e., domesticated cultivars from intensive production) to the attack of this potentially invasive pest.

2. Materials and methods

2.1. Herbivore

A. ludens is a polyphagous pest of citrus (*Citrus* sp.), mango (*Mangifera indica* L.), and a number of additional fruit crops (Birke et al., 2013). This fly is widely distributed in tropical and subtropical regions of Mexico and Central America (Hernández-Ortíz and Aluja, 1993), and exhibits a high degree of physiological and behavioral plasticity which increases its adaptability to varying environmental conditions (Díaz-Fleischer and Aluja, 2003a,b). This pest also has enormous capacity to move over long distances (Birke et al., 2013). *A. ludens* could therefore easily expand its distribution to temperate areas under a climate change/global warming scenario, attacking commercially grown apples.

A. ludens pupae were obtained from field-collected larvae stemming from *Citrus aurantium* L. ('bitter orange') collected in Dos Ríos, Veracruz, Mexico. Pupae in the range of 15–25 mg were stored in 500 ml plastic containers with moist vermiculite. Pupae were transported by air from Mexico to a Level II quarantine laboratory at the Swiss Federal Research Station Agroscope, Changins-Wädenswil in Wädenswil (Switzerland) and reared to adulthood in two Plexiglas cages (50 × 70 × 50 cm) with small fans located in the base of each cage for aeration. Environmental conditions were maintained at 27 ± 1 °C, 70 ± 10% RH, and LD 12:12 h photoperiod provided by 36 W Philips® daylight bulbs. From emergence, adult flies were given *ad libitum* access to water and a 3:1 mixture of sugar (Feinkristallzucker, Coop® Basel, Zürich, Switzerland) and protein hydrolyzate (Biomedicals, LLC, lot. No 1414K, France). In Mexico, exactly the same procedures were followed, except that sources of sugar (COSTCO®, Mexico) and protein hydrolysate (Greif Bros. Corp., Delaware, Ohio, US), varied.

2.2. Apple cultivars

In Switzerland, we used four locally-grown cider apple cultivars and eight commercial dessert cultivars ('Milwa', 'Fuji', 'Gala', 'Golden Delicious (hereafter Golden)', 'Braeburn', 'Elstar', 'Topaz' and 'Idared'). Apples were harvested between August and October 2009, depending on cultivar, and kept in environmentally controlled rooms at 3 °C (cider cultivars) and 1 °C (dessert cultivars). Florida 4920 'Marsh' grapefruit, an *A. ludens* preferred host in Mexico, were obtained from a local supermarket and maintained at 2–4 °C during a 2-day period prior to testing.

In Mexico, apples are commercially grown over 61,552 ha in the northern states of Chihuahua, Durango, and Coahuila, and in the central state of Puebla which harbors 14% of the total cultivated surface in the country. Puebla is the area with the highest risk of being invaded by *A. ludens* due to its proximity to tropical citrus growing regions where *A. ludens* populations are extremely high (e.g., Martínez de la Torre and Tlapacoyan both in the neighboring state of Veracruz). This highland apple-growing region also harbors the largest surfaces planted with cider apples in Mexico (SIAP, 2012). In the localities of González Ortega and La Fragua, Puebla we collected the locally-grown cider-apple cultivars 'San Juanera', 'Rayada', 'Chipona' and 'Española', and commercial dessert cultivars 'Starking', 'Red Delicious' and 'Golden' fruit was stored at 4 °C before being used in experiments. Storage temperatures of Swiss and Mexican apples were determined based on shelf life requirements (Kellerhals, 2009).

Experimental apples and grapefruit were washed with liquid detergent (Manella-Nature, Coop® Basel, Zürich, Switzerland; Neutral-liquid soap, Grisi®, Mexico), rinsed with water, dried, and maintained overnight at 22 °C before exposure to mated female flies for a 72 h period. Three days after the 3-day exposure period a 0.5% (wt/wt) sodium benzoate solution (VW®, Merck, Switzerland; Baker, Phillipsburg, US in the case of Mexico) was applied to fruit using a cloth to avoid fungal growth and premature fruit decay.

2.3. Fruit parameters

Samples of 15 (Switzerland) or 10 (Mexico) apples of each cultivar were selected at two sampling dates and 15 grapefruit at one sampling date were selected to measure the following features which have been shown to influence oviposition by *A. ludens* (Berrigan et al., 1988; Díaz-Fleischer and Aluja, 2003a): fruit weight, size, firmness, and sugar content. Apples sampled in Switzerland were also subjected to phenolic compound analyses. Unfortunately we could not perform the same analyses in the same Swiss laboratory with the Mexican fruit due to phytosanitary restrictions and we acknowledge this as a weakness in our study.

2.3.1. Fruit weight

Fruit was weighed using a precision scale (Mettler PC2200 DeltaRange, Mettler-Toledo, Greifensee, Switzerland; Ohaus, TP4KD, US in the case of Mexico).

2.3.2. Size

Fruit size was determined by measuring diameter (at the widest point) and height for each fruit using a vernier (dialMax, Kunststoffwerk Buchs, Buchs SG, Switzerland; Baco, Mexico, in the case of Mexico).

2.3.3. Fruit flesh firmness

Fruit flesh firmness was determined by performing two equatorial punctures per fruit on the sunny (i.e., the reddish side of the fruit) and shady side (i.e., opposite to the sunny side) using an UP-PE01 penetrometer (Umweltanalytische Produkte GmbH, Ibbenbüren, Germany; SPER scientific, 300051, Hong Kong, China in the case of Mexico) with a 1 cm^2 aluminum probe.

2.3.4. Sugar content

A cut was made on both equatorial sides of the fruit and a piece of each fruit was pressed to obtain a drop of juice. Sugar content was then measured using a hand-held digital refractometer (ATAGO ART-RE101, Tecfrut AG, Au/Wädenswil, Switzerland; ATAGO, PAL-1, Tokyo, Japan in the case of Mexico).

2.3.5. Total content of phenolic compounds and pattern

Swiss apples were analyzed for total content of phenolic compounds after being removed from the cold room at the beginning of the experiment. Three independent samples of five apples of each cultivar were sliced into pieces and the core was removed. Slices were immediately frozen in liquid nitrogen and crushed in a dry ice mill. After grinding to a fine powder, samples were stored at −20 °C until extraction. Total phenolic content was quantified by the Folin–Ciocalteu assay with results expressed as mg catechin equivalents per kg fresh matter. The identities of phenolic compounds were determined by UHPLC–MS analysis (Ceymann et al., 2011).

To estimate the influence of high temperature storage (matching the tropical conditions of the experiment) on phenolic content, samples of five apples each were taken as described above, before and after apples had been stored for 14 days in the experimental room at 27 °C.

2.4. Experimental set up

Based on a completely randomized design, five replicates for each of 13 treatments consisting of 12 apple cultivars and one control fruit (grapefruit) were set up. Each experimental unit consisted of a cage containing six fruit and 12 gravid females aged 12 days post-emergence. Cages were 25 × 15 × 30 cm with metal frame and covered with a 21 cm tubular net bandage (textile elasticity, STÜLPA®, Paul Hartmann AG, Heidenheim, Germany; 30 × 30 × 30 cm Plexiglas cages in the case of Mexico) inside which a 25 × 30 × 10 cm plastic tray with six apples was placed. Flies were given *ad libitum* access to food and water. Apples and grapefruits were exposed to flies for a 72-h period.

2.5. Oviposition parameters and egg laying propensity

One randomly selected fruit per treatment and replicate was used to determine oviposition depth, number of eggs per clutch and total number of eggs per fruit. Each fruit was peeled, sliced and oviposition sites were located and dissected under a stereomicroscope (Leica-Wild, MRZ, Heerbrugg, Switzerland; Nikon, SMZ1500, Tokio, Japan in the case of Mexico). Three fruit per treatment were dissected after a 5-day period, one fruit after an 8-day period and the last after an 11-day period. In each case oviposition depth and egg number per clutch and per fruit were quantified.

2.6. Infestation levels

Following the 72-h exposure period, fruit was removed and placed individually in 1 l (apples) or 4 l plastic cups (larger fruit such as grapefruit and 'Golden' apples) containing a layer of vermiculite in the base of each container. Development time was determined by daily sampling of pupae that emerged from the fruit. Fruit were maintained until they had decomposed and larvae and pupae were recovered. Each fruit was then dissected to ascertain if any larvae had pupated or died within fruit. Recovered pupae were transferred to small plastic containers (50 ml capacity) with damp vermiculite and individually weighed 4 days after pupation.

2.7. Longevity

In Mexico the longevity of adults (i.e., mean number of days that adults survived) was determined using pairs (male and female) that were randomly selected from cultivars exhibiting high infestation: 'Marsh' grapefruit (N = 24 pairs), 'Golden' (N = 6 pairs) and 'Española' (N = 12 pairs) apples. In the case of the other cultivars that yielded much smaller numbers of adult flies, all individuals that emerged were used ('Chipona' = 6, 'Rayada' = 1, 'Red Delicious' = 1, individuals, respectively). No adult fly was recovered from 'San Juanera' apples. Pairs were held in ventilated 1 l plastic boxes and were given *ad libitum* access to water and a 3:1 mixture of sugar and hydrolyzed protein. We note that we were unable to measure this parameter in Switzerland due to national phytosanitary restrictions.

2.8. Protein content in hemolymph and immune response

At 4 days after pupation, pupae recovered in Switzerland were individually weighted using an analytical scale. We randomly selected a sample of pupae from each apple cultivar and grapefruit for protein and phenoloxidase analyses. When only few pupae were recovered from a particular cultivar, all pupae were used. Hemolymph samples were obtained by pushing the tip of a drawn-out capillary tube (Minicaps 100 μl, Glaswerk Wertheim, Wertheim, Germany) into each pupa. Each hemolymph sample was immediately transferred to a 1.5 ml Eppendorf tube containing 200 μl of ice-cold phosphate buffered saline (PBS) (pH 7.5), mixed with a vortex, and frozen at −20 °C.

2.8.1. Protein content

Protein content was measured using SIGMA Bicinchoninic Acid Protein Assay Kit (BCA1 and B9643) as described by Contreras-Garduño et al. (2007). Optical density measurements were performed at 37 °C using a 96 U Greiner bottom transparent polystyrene plate and a 562 nm filter in an Infinite® M200 Spectrophotometer (Tecan Schweiz AG, Männedorf, Switzerland). Measurements were recorded five times every 5 min and the average reading was calculated.

2.8.2. Phenoloxidase activity

The activation of phenoloxidase that convert diphenols into quinones that subsequently polymerize melanin is a commonly used indicator of insect immune response (Söderhäll and Cerenius, 1998). PO activity was determined by taking a 25 μl volume of the hemolymph sample with a concentration of 10 μg/μl of protein that was added to 150 μl of PBS and mixed on a 96 well microplate with 25 μl L-DOPA (Contreras-Garduño et al., 2007). Optical density measurements were performed at 37 °C using a 490 nm filter in an Infinite® M200 Spectrophotometer (Tecan Schweiz AG, Männedorf, Switzerland). Measurements were recorded 13 times every 5 min

Table 1
Physicochemical characteristics of fruit of different apple cultivars and grapefruit in Switzerland.

Cultivar	Weight (g) (SE)	Fruit flesh firmness (kg/cm^2) (SE)	Sugar content (°Brix) (SE)	TPC[1] (g/kg) (SE)	TPC change at high temperature storage	
					Proportion	Paired *t*-test (*P*)
Grauer Hordapfel	76.7 (8.96)	5.67 (0.30)	14.9 (0.37)	3.41 (0.096)	+0.21	0.097, n.s.
Engishofer	72.2 (3.14)	8.92 (0.17)	13.8 (0.43)	3.25 (0.065)	+0.03	0.165, n.s.
Bohnapfel	78.1 (5.32)	7.46 (0.29)	13.1 (0.28)	3.25 (0.052)	+0.24	0.192, n.s.
Schneiderapfel	136.2 (7.85)	5.95 (0.11)	12.5 (0.12)	2.39 (0.037)	+0.01	0.849, n.s.
Fuji	177.2 (10.88)	6.34 (0.13)	14.1 (0.37)	1.39 (0.028)	+0.16	0.195, n.s.
Idared	190.6 (10.52)	5.99 (0.15)	14.2 (0.48)	1.30 (0.077)	+0.09	0.181, n.s.
Braeburn	211.2 (7.57)	8.04 (0.24)	15.3 (0.12)	1.14 (0.026)	−0.05	0.788, n.s.
Elstar	152.8 (2.66)	5.82 (0.21)	15.2 (0.45)	1.20 (0.033)	−0.17	0.164, n.s.
Topaz	148.3 (1.55)	6.72 (0.23)	13.2 (0.31)	1.07 (0.023)	−0.12	0.383, n.s.
Milwa	181.1 (4.92)	6.74 (0.15)	14.6 (0.38)	1.10 (0.038)	−0.07	0.073, n.s.
Gala	186.4 (3.14)	6.55 (0.13)	13.4 (0.29)	1.25 (0.046)	+0.04	0.677, n.s.
Golden	211.1 (9.44)	4.96 (0.23)	13.9 (0.72)	1.21 (0.041)	−0.11	0.103, n.s.
Grapefruit	350.4 (7.61)	7.67 (0.37)	10.9 (0.32)			

[1] Total phenolic content (TPC, Folin–Ciocalteu) in g catechin equivalents per kg fruit fresh mass.
n.s. not significant.

and the mean value was calculated. Tyrosinase (Sigma T-7755 2000 U/mg) was used as an external standard.

2.9. Statistical analyses

Results related to apple flesh firmness, weight, size, sugar content and total content of phenolic compounds measurements were each subjected to one-way analysis of variance (ANOVA) with apple cultivar as explanatory variable. The effect of storage temperature on phenolic content was analyzed by paired *t*-tests of samples taken before and after storage. Infestation level (number of pupae per fruit), number of eggs per clutch and fruit, pupal weight, developmental time (egg–pupa), adult longevity, protein and phenoloxidase content of pupae were also subjected to a one-way ANOVA. All data stemming from pupae (pupal weight, developmental time, protein content and phenoloxidase activity) were averaged prior to analysis to avoid bias due to high variability in sample size (range of 1–84 pupae) across apple cultivars. Post-hoc Tukey HSD tests were applied for means separation. Immature mortality was estimated by calculating the percentage of insects that developed from egg to pupa based on the number of pupae emerged and the estimated number of eggs in the total sample. Mortality between cultivars was compared with *z*-tests. The association of fruit characteristics and phenolic content, with fly developmental parameters and infestation levels, respectively, were examined using Pearson's correlation analyses. If a test produced a non-significant result, "n.s." is reported. All data were normally distributed and displayed constant variance. Statistical analyses were performed using XLStat Pro V2011.204 (Addinsoft, Andernach, Germany for results obtained in Switzerland) and Statistica Version 7 (Statsoft, US) for results obtained in Mexico.

3. Results

3.1. Fruit parameters and phenolic compounds

In Switzerland, apple cultivars and grapefruit exhibited significant differences in fruit weight (ANOVA, $F_{12, 129} = 107.7$, $P < 0.0001$), firmness (ANOVA, $F_{12, 129} = 24.2$, $P < 0.0001$) and sugar content (ANOVA, $F_{12, 129} = 107.7$, $P < 0.0001$) (Table 1). However, the biotic responses of *A. ludens* were not significantly correlated with fruit weight, firmness or sugar content (Pearson's correlations, n.s.). In Mexico, apple cultivars and grapefruit also differed significantly in fruit weight (ANOVA, $F_{7, 72} = 145.62$, $P < 0.0001$), firmness (ANOVA, $F_{7, 72} = 8.847$, $P < 0.0001$) and sugar content (ANOVA, $F_{7, 72} = 13.07$, $P < 0.0001$) (Table 2). Similarly, the biotic responses of *A. ludens* were not correlated with any of these characteristics of Mexican apples (Pearson's correlations, n.s.).

Total phenolic content varied significantly among apple cultivars tested in Switzerland (ANOVA, $F_{11, 35} = 343.9$, $P < 0.0001$) (Table 1). The highest values were present in the cider cultivars 'Grauer Hordapfel', 'Engishofer' and 'Bohnapfel' followed by slightly, but significantly, lower values in 'Schneiderapfel' (Table 1). In contrast, the dessert apple cultivars all exhibited relatively low values of phenolic compounds, with 'Fuji' showing the highest values and 'Topaz' and 'Milwa' the lowest values (Table 1). Total phenolic content did not differ significantly in apples sampled before and after high temperature storage (Table 1, paired *t*-tests).

Cultivars differed significantly across the four groups of phenolic compounds analyzed by UHPLC–MS (ANOVA, flavan-3-ols: $F_{11, 35} = 75.9$, $P < 0.0001$; phenolic acids: $F_{11, 35} = 235.6$, $P < 0.0001$; dihydrochalcons: $F_{11, 35} = 384.0$, $P < 0.0001$; flavonols: $F_{11, 35} = 12.6$, $P < 0.0001$) (Fig. 1a).

3.2. Herbivore responses

3.2.1. Oviposition parameters and egg laying propensity

Clutch size was found to differ significantly among host fruit (ANOVA, $F_{12, 59} = 3.74$, $P < 0.001$) (Fig. 2a) with the largest fruit (grapefruit, 'Golden') receiving the largest mean egg clutches (Fig. 2a, significant grouping for grapefruit). The total number of eggs per fruit, however, was not influenced by cultivar (ANOVA, $F_{12, 65} = 0.453$, $P = 0.933$). Clutch size and eggs per fruit did not show a significant correlation with the type of phenols present (Pearson's correlations, n.s.). Clutch size (ANOVA, $F_{7, 32} = 1.46$, $P = 0.134$) and oviposition depth (ANOVA, $F_{7, 32} = 1.46$, $P = 0.134$) in the Mexican fruit did not differ significantly. Oviposition depth in Switzerland varied significantly among host fruit (ANOVA, $F_{12, 193} = 2.43$, $P = 0.006$) (Fig. 2b) but was not correlated with type of phenols present (Pearson's correlations, n.s.).

3.2.2. Infestation level

In Switzerland, infestation level, measured as mean number of pupae per fruit and total number of pupae per treatment (i.e., cultivars), differed significantly among host fruit (ANOVA, $F_{12, 323} = 33.5$, $P < 0.0001$) (Fig. 1b) and was negatively correlated with total phenolic content ($r = -0.680$, Table 3). Importantly, no pupae developed in locally-grown 'Engishofer' and 'Grauer Hordapfel' and only very few pupae emerged from locally-grown 'Schneiderapfel' and 'Bohnapfel' cider cultivars, or the dessert apple 'Fuji' (Fig. 1b and c). Among the single compounds analyzed, procyanidin B2 exhibited a significant negative relationship ($r = -0.579$) with the mean number of pupae that developed per fruit (Table 3).

In Mexico, infestation levels also differed significantly (ANOVA, $F_{7, 72} = 31.21$, $P < 0.0001$) (Fig. 3a and b). No pupae developed in the locally-grown cultivar 'San Juanera' and only very few were obtained from 'Rayada', 'Starking', 'Red Delicious' (local breed) and 'Chipona' when compared to 'Española' and 'Golden' (Fig. 3a and b).

Table 2
Physicochemical characteristics of fruit of different apple cultivars and grapefruit in Mexico.

Cultivar	Weight (g) (SE)	Fruit flesh firmness (*N*) (SE)	Sugar content (°Brix) (SE)
San Juanera	20.4 (0.85)	8.75 (0.59)	14.9 (0.37)
Red Delicious	72.1 (7.61)	10.54 (0.75)	13.8 (0.43)
Rayada	87.1 (7.34)	11.91 (0.34)	13.1 (0.28)
Golden	90.2 (3.16)	8.39 (0.39)	12.5 (0.12)
Española	105.7 (6.28)	10.26 (0.59)	14.1 (0.37)
Starking	121.8 (7.39)	7.37 (0.37)	14.2 (0.48)
Chipona	123.3 (6.74)	6.84 (0.78)	15.3 (0.12)
Elstar	152.8 (2.66)	5.82 (0.21)	15.2 (0.45)
Grapefruit	335.74 (14.69)	10.14 (0.64)	10.9 (0.32)

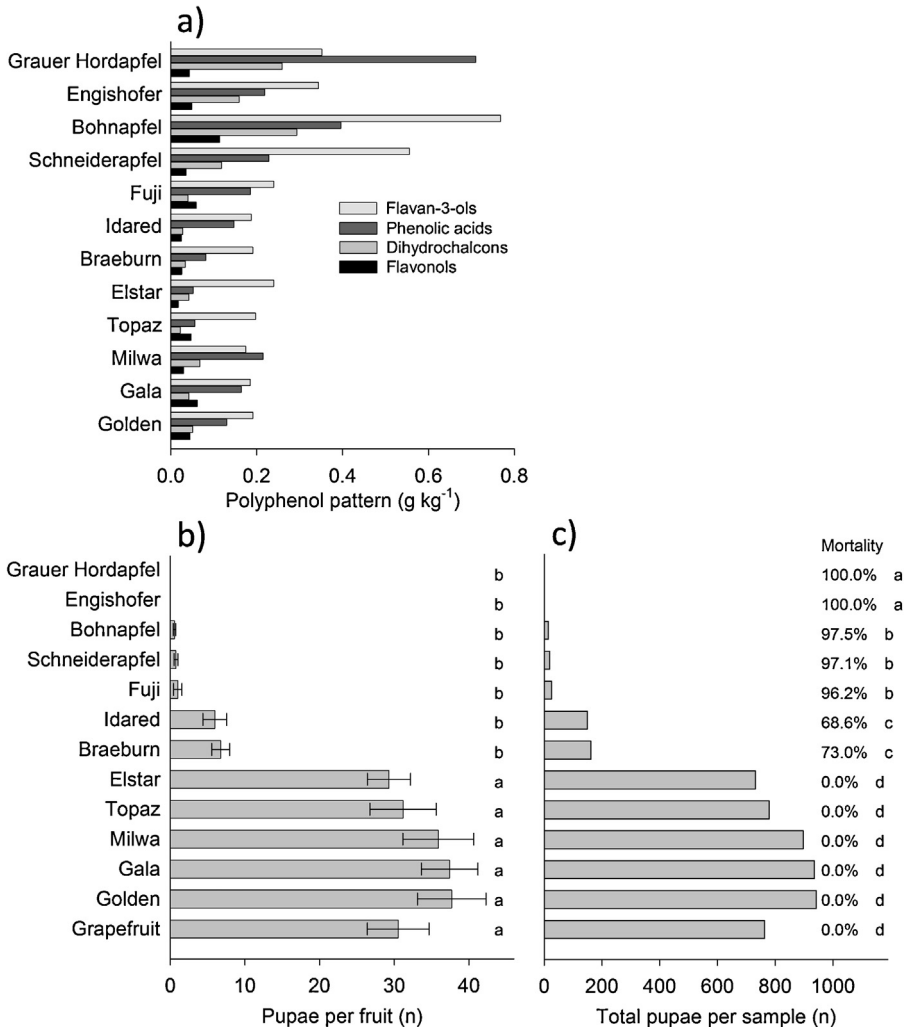

Fig. 1. Phenolic compounds pattern of the four main groups analyzed by UHPLC–MS in mg per kg fresh matter of different apple cultivars (a), as related to infestation (pupae per fruit and sample) by *Anastrepha ludens* (b and c) and mortality of eggs and larvae (c). Means ± SE; different letters at columns indicate significant differences of Tukey HSD post-hoc tests (b) or *z*-tests for mortality (c).

In the case of 'Golden' studied in both Switzerland and Mexico, results show that in both countries it was the most infested apple cultivar of all the ones tested. We note however, that the "Swiss" 'Golden' yielded many more pupae than the "Mexican" 'Golden'.

In Switzerland, mortality of eggs and larvae was significantly higher in the apples with high total phenolic content ($r = 0.728$, Table 3), whereas all eggs and larvae died during development in the locally-grown cultivars 'Engishofer' and 'Grauer Hordapfel'. Mortality in 'Schneiderapfel', 'Bohnapfel' and 'Fuji' was also very high (Fig. 1c), followed by 'Idared' and 'Braeburn', as calculated by the estimated number of eggs in the samples and the emergence of larvae prior to pupation. Among the phenolic groups analyzed, the flavan-3-ols (total: $r = 0.594$; procyanidin B2: $r = 0.614$) and the dihydrochalcons ($r = 0.597$) exhibited a significant positive relationship with mortality (Table 3).

Developmental time in Switzerland varied significantly between fruit (ANOVA, $F_{10, 218} = 92.4$, $P < 0.0001$) (Fig. 2c). Larvae reared in grapefruit and 'Golden' apples developed the fastest. The first *A. ludens* larvae reared in grapefruit pupated after 19 days, and in 'Golden' apples after 23 days. Apple cultivars with higher phenolic content ('Schneiderapfel', 'Fuji', 'Bohnapfel' and 'Braeburn') produced pupae with the longest developmental times, almost double the values observed in grapefruit (Fig. 2c). In addition, first instar larvae developing in 'Bohnapfel', 'Engishofer' and 'Fuji' apples were observed to be covered consistently with red bands along the body suggesting probable toxic effects of the pulp. In the case of the locally-grown 'Schneiderapfel', mean larval development time was almost twice as long as in the natural host grapefruit (67 ± 2.1 vs 36.4 ± 0.8 days [±SE], respectively). In addition, many larvae were unable to complete metamorphosis as they transformed into deformed/malformed pupae (Fig. 4) or hardened larvae that did not complete metamorphosis (i.e., did not transform into pupae). Development time was positively correlated with the content of procyanidin B1 ($r = 0.662$, Table 3).

In Mexico, development time also varied significantly among host fruit (ANOVA, $F_{6, 27} = 6.126$, $P = 0.0004$) (Fig. 3c). Larvae reared in grapefruit also developed the fastest followed by larvae reared in 'Golden' (Fig. 3c). As found for cider apples in Switzerland, larvae reared in locally-grown 'Chipona' apples were also observed to have red stripes along the body segments.

In Switzerland, pupal weight differed significantly among host fruit (ANOVA, $F_{10, 218} = 42.1$, $P < 0.0001$) (Fig. 2d). Pupae that had developed in locally-grown 'Bohnapfel' and 'Schneiderapfel' weighed the least (Fig. 2d). Pupae obtained from grapefruit attained the greatest weight (23.5 ± 0.39 mg) followed by pupae from 'Milwa' (17.7 ± 0.38 mg) and 'Golden' (15.6 ± 0.35 mg) apples (Fig. 2d). Pupal weight was significantly higher in apples with lower total content of phenolic compounds than for those with higher content ($r = -0.823$, Table 3). Among the phenolic groups identified the total flavan-3-ols ($r = -0.819$) and the dihydrochalcons ($r = -0.643$) showed significant negative relationships with pupal weight (Table 3).

In Mexico, pupal weight varied significantly between host fruit (ANOVA, $F_{6, 27} = 9.76$, $P < 0.0001$), but did not differ among apple cultivars (Fig. 3d), and adult emergence differed significantly among host fruit (ANOVA, $F_{3, 63} = 12.4$, $P < 0.0001$) (Fig. 3e). Adult emergence was highest from pupae reared on grapefruit (705 adults/1409 pupae = ca 50%) followed by 'Española' (60 adults/217 pupae = 28%), 'Golden' (25 adults/258 pupae = 10%) and 'Chipona' (6 adults/63 = 10%) cultivars.

Adult longevity did not differ significantly among apple cultivars, but did differ between apples and grapefruit (ANOVA, $F_{4, 28} = 3.43$, $P < 0.0001$).

In Switzerland, pupal hemolymph protein content differed significantly among cultivars (ANOVA, $F_{10, 208} = 3.43$, $P < 0.0001$) (Fig. 2e). Pupae obtained from locally-grown 'Bohnapfel' and 'Schneiderapfel' had the lowest protein contents; pupae obtained from 'Milwa' and 'Gala' had the highest values (Fig. 2e). Protein content exhibited a strong negative relationship with total phenolic compounds content ($r = -0.825$), the total flavan-3-ols ($r = -0.820$) and the dihydrochalcons ($r = -0.690$) (Table 3).

Pupal hemolymph phenoloxidase differed markedly among host fruit (ANOVA, $F_{10, 207} = 3.23$, $P < 0.001$) (Fig. 2f) and correlated significantly with low total phenolic content ($r = -0.735$, Table 3).

Table 3
Pearson correlation coefficients for apple phenolic compounds and herbivore parameters.

	Infestation (pupae per fruit)	Mortality (egg, larva)	Development time (egg, larva)	Pupal weight	Pupal hemolymph protein	Pupal hemolymph phenoloxidase
Total phenolic content (TPC)	**−0.680** ($P = 0.015$)	**0.728** ($P = 0.007$)	0.623 ($P = 0.054$)	**−0.823** ($P = 0.003$)	**−0.825** ($P = 0.003$)	**−0.735** ($P = 0.015$)
Flavan-3-ols	−0.565 ($P = 0.056$)	**0.594** ($P = 0.042$)	0.589 ($P = 0.073$)	**−0.819** ($P = 0.004$)	**−0.820** ($P = 0.004$)	**−0.680** ($P = 0.030$)
Catechin	−0.493 ($P = 0.103$)	0.511 ($P = 0.089$)	0.621 ($P = 0.055$)	**−0.777** ($P = 0.008$)	**−0.715** ($P = 0.020$)	**−0.738** ($P = 0.015$)
Epicatechin	−0.474 ($P = 0.120$)	0.495 ($P = 0.102$)	0.534 ($P = 0.112$)	**−0.733** ($P = 0.016$)	**−0.743** ($P = 0.014$)	−0.575 ($P = 0.082$)
Procyanidin B1	−0.548 ($P = 0.065$)	0.572 ($P = 0.052$)	**0.662** ($P = 0.037$)	**−0.862** ($P = 0.001$)	**−0.807** ($P = 0.005$)	**−0.788** ($P = 0.007$)
Procyanidin B2	**−0.579** ($P = 0.049$)	**0.614** ($P = 0.034$)	0.540 ($P = 0.107$)	**−0.794** ($P = 0.006$)	**−0.820** ($P = 0.004$)	**−0.636** ($P = 0.048$)
Phenolic acids	−0.479 ($P = 0.116$)	0.540 ($P = 0.070$)	0.560 ($P = 0.092$)	−0.503 ($P = 0.138$)	−0.615 ($P = 0.058$)	**−0.654** ($P = 0.040$)
Chlorogenic acid	−0.417 ($P = 0.178$)	0.476 ($P = 0.118$)	0.437 ($P = 0.206$)	−0.350 ($P = 0.321$)	−0.481 ($P = 0.159$)	−0.514 ($P = 0.128$)
Coumaroylquinic acid	−0.523 ($P = 0.081$)	0.568 ($P = 0.054$)	0.440 ($P = 0.203$)	−0.522 ($P = 0.122$)	−0.482 ($P = 0.158$)	−0.505 ($P = 0.137$)
Dihydrochalcons	−0.544 ($P = 0.068$)	**0.597** ($P = 0.041$)	0.476 ($P = 0.165$)	**−0.643** ($P = 0.045$)	**−0.690** ($P = 0.027$)	−0.550 ($P = 0.099$)
Phloridzin	−0.494 ($P = 0.102$)	0.543 ($P = 0.068$)	0.458 ($P = 0.183$)	−0.520 ($P = 0.124$)	**−0.700** ($P = 0.024$)	−0.518 ($P = 0.125$)
Phloretin-xyloglucoside	−0.512 ($P = 0.089$)	0.561 ($P = 0.058$)	0.474 ($P = 0.167$)	**−0.662** ($P = 0.037$)	**−0.679** ($P = 0.031$)	−0.551 ($P = 0.099$)
Flavonols	−0.222 ($P = 0.488$)	0.294 ($P = 0.354$)	0.284 ($P = 0.427$)	−0.428 ($P = 0.218$)	−0.539 ($P = 0.108$)	−0.413 ($P = 0.235$)
Quercetin-galactoside, and glucoside	−0.281 ($P = 0.376$)	0.371 ($P = 0.235$)	0.407 ($P = 0.243$)	−0.442 ($P = 0.200$)	−0.436 ($P = 0.208$)	−0.492 ($P = 0.149$)
Rutin	−0.213 ($P = 0.506$)	0.281 ($P = 0.376$)	0.395 ($P = 0.258$)	−0.431 ($P = 0.214$)	−0.480 ($P = 0.160$)	−0.505 ($P = 0.136$)
Quercetin-rhamnoside	−0.089 ($P = 0.782$)	0.120 ($P = 0.711$)	0.001 ($P = 0.998$)	−0.289 ($P = 0.419$)	−0.538 ($P = 0.108$)	−0.156 ($P = 0.667$)

Values in bold indicate significant correlations ($P < 0.05$).

Pupae obtained from locally-grown 'Bohnapfel' and 'Schneiderapfel' exhibited the lowest phenoloxidase content; pupae obtained from 'Golden', 'Topaz' and 'Milwa' had the highest values (Fig. 2f). Phenoloxidase content was negatively correlated with flavan-3-ols ($r = -0.680$) and total phenolic acid content ($r = -0.654$, Table 3).

4. Discussion

Studies performed in both Switzerland and Mexico revealed significant differences in *A. ludens* offspring performance among apple cultivars despite the fact that female egg-laying propensity in the same cultivars did not differ. In both cases, locally-grown apple cultivars (e.g., 'Bohnapfel', 'Schneiderapfel' [Switzerland] or 'San Juanera' and 'Rayada' [Mexico]) significantly reduced *A. ludens* infestation and offspring viability when compared to widely-grown, commercial apple cultivars (e.g., 'Golden'). Phenolic compounds are known to be involved in plant defense against herbivores (Treutter, 2005, 2010). In insects, they can cause degenerative lesions in midgut tissues (Lindroth and Peterson, 1988), bind to taste receptor cells thus making flavors unpalatable (Isman, 2002), and can bind to proteins and digestive enzymes inhibiting protein digestion (Schopf, 1986; Felton et al., 1992). Therefore phenolics, may act as toxins, feeding deterrents or digestion inhibitors. These findings are consistent with our observations on the relationship between total phenolic content and high larval mortality, low protein content in hemolymph and low pupal weight in *A. ludens*. In addition, incomplete metamorphosis (i.e., hardened larvae unable to transform into pupae) and pupal malformations observed in the 'Bohnapfel', 'Schneiderapfel' and 'Fuji' cultivars (Fig. 4), may have been related to the ability of some phenolic compounds to inhibit the activity of phenoloxidase, a key enzyme in the molting process (Wang et al., 2005). A deficiency of this enzyme in insect larvae can result in malformed pupae and adults, together with increased mortality (Arakane et al., 2005). Thus, our prediction that variation in fruit phenolic compounds would reduce the capacity of the Mexican fruit fly to invade apple agroecosystems in areas affected by global warming is supported.

Results from the study in Switzerland demonstrated that highly resistant cultivars such as the locally-grown 'Grauer Hordapfel' or 'Engishofer' that completely inhibited *A. ludens* larval development, had a significantly higher total content of phenolic compounds than susceptible cultivars such as the commercially grown 'Golden', 'Gala' or 'Milwa', which yielded large numbers of larvae. Interestingly, the cultivar 'Fuji', that contained intermediate amounts of phenolic compounds, also proved highly deleterious to larvae. In Mexico, although we did not quantify the content of phenolic compounds, results were consistent with those in Switzerland as we also found that locally-grown cultivars such as 'San Juanera', 'Rayada' and 'Chipona' were highly resistant to the attack of the potentially invasive herbivore.

The group of flavan-3-ols appeared to be of major importance as these compounds significantly influenced all biotic parameters measured. The group of dihydrochalcons was also important, as it was associated with increased mortality, depressed pupal weight and hemolymph protein content. Phenolic acids were correlated with a reduction in pupal hemolymph phenoloxidase, whereas the presence of flavonols did not correlate significantly with any of the parameters measured despite the fact that the latter compounds, particularly quercetin and rutin, have been shown to cause high egg and larvae mortality in insects and inhibit phenoloxidase activity (Mallikarjuna et al., 2004; Wang et al., 2005). Overall, high total phenolic compounds content was significantly correlated with all parameters measured with the exception of development time; in this case only Procyanidin B2 was positively correlated. Importantly, larvae stemming from grapefruit and from apples with very low phenolic compounds content ('Golden') developed

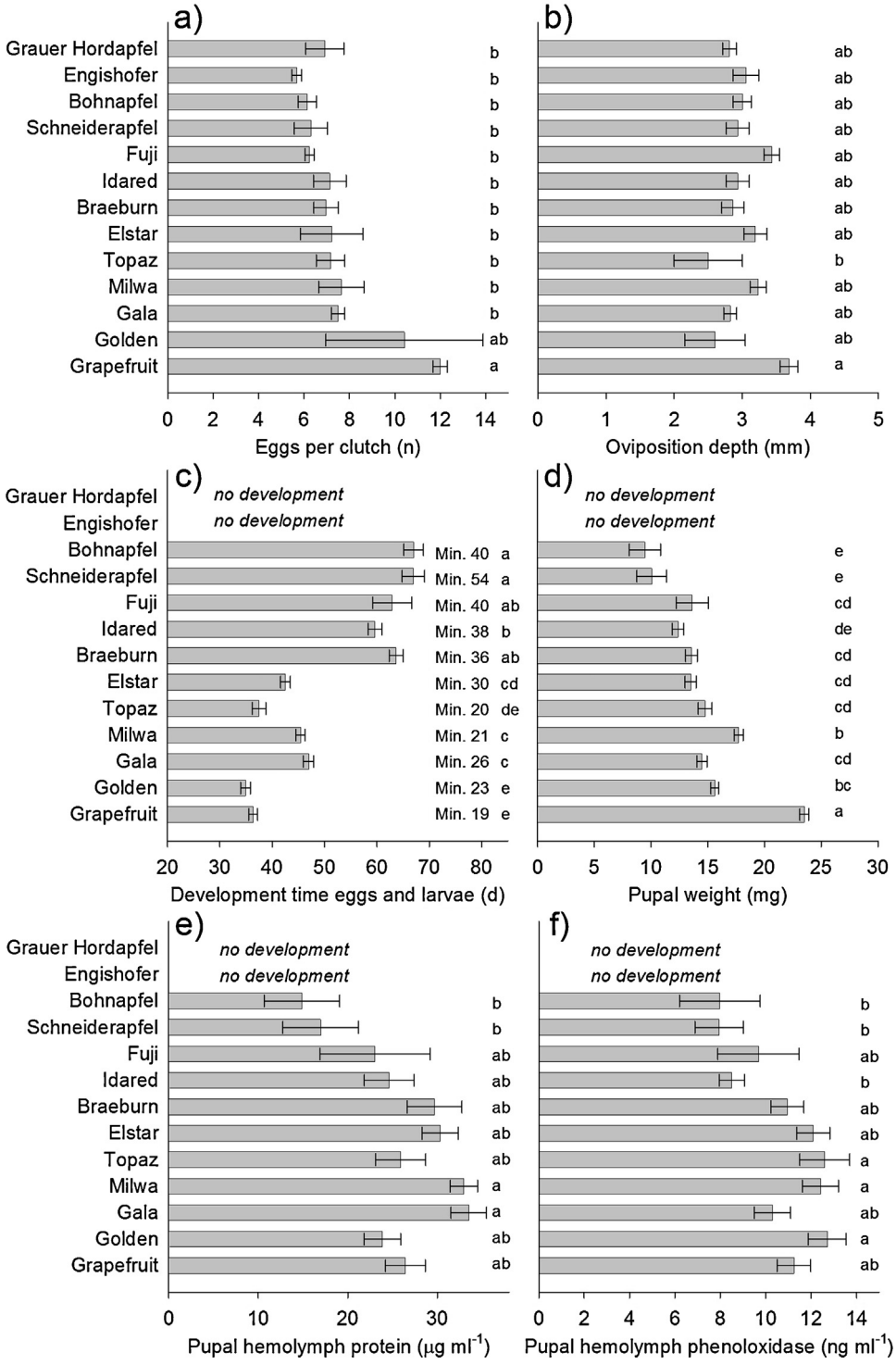

Fig. 2. Response in Switzerland by *Anastrepha ludens* to different *Malus* × *domestica* cultivars and *Citrus paradisi*, reflected by oviposition parameters (a and b), development time of eggs/larvae (c), pupal weight (d), pupal hemolymph protein (e) and phenoloxidase (f) as measures of physiological constitutions and immune activity, respectively. Means ± SE; different letters at columns indicate significant differences of Tukey HSD post-hoc tests.

significantly faster and were heavier than those developing in cultivars with high phenolic content, potentially granting emerging adults better chances for survival and reproduction.

Our results, are consistent with previous research reporting a positive correlation between the total content of phenolic compounds in cultivars of host fruit such as crab apples (*Malus* sp.), guava (*Psidium guajava* L.) and bitter gourd (*Momordica charantia* L.), and its resistance to infestation of the flies *Rhagoletis pomonella* (Walsh), *Bactrocera correcta* (Bezzi) and *Bactrocera cucurbitae* (Coquillet), respectively (Pree, 1977; Jalaluddin and Sadakathulla, 1999; Gogi et al., 2010). Fly larvae that fed on cultivars with high levels of phenolic compounds died or failed to develop, adult emergence was depressed and adults were smaller. Hence, it is clear that correlates of fitness of some pestiferous tephritid flies decrease in the presence of high content of phenolic compounds. Breeding programs aimed at increasing palatability to humans have diluted

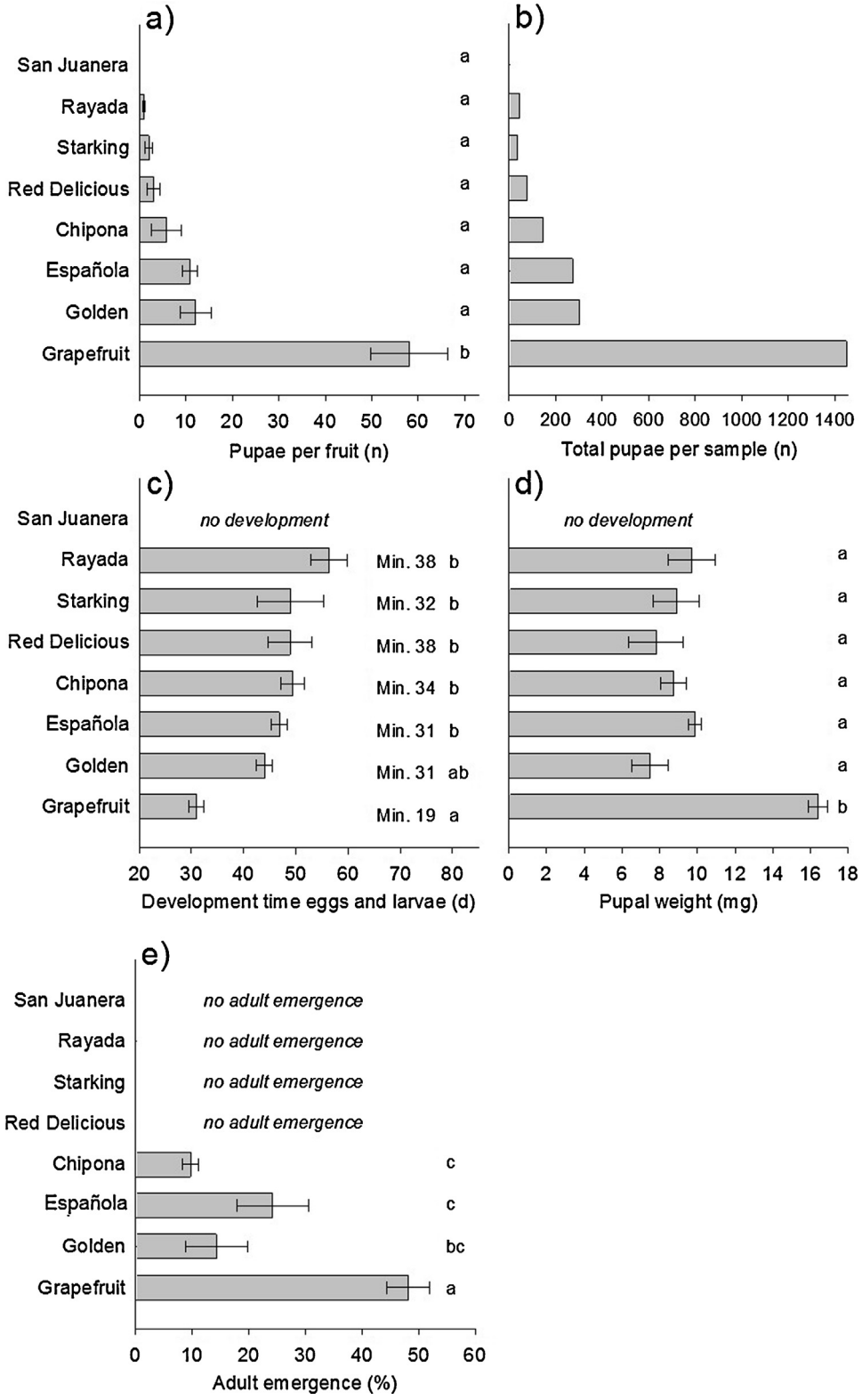

Fig. 3. Response in Mexico by *Anastrepha ludens* to different Mexican apple cultivars and grapefruit, reflected by infestation level (pupae per fruit and sample) (a and b), development time of eggs/larvae (c), pupal weight (d) and adult emergence (e). Means ± SE; different letters at columns indicate significant differences of Tukey HSD post-hoc tests.

genetic diversity and removed many defensive secondary metabolites, among them phenolic compounds, from widely-grown apple cultivars (Aluja and Mangan, 2008; Kellerhals, 2009; Kumar et al., 2012). Therefore, some of the compounds that were bred out in the past may have to be bred back to fruit to increase their resilience to invasive pests and diseases under current global climate change scenarios (Gogi et al., 2010; Treutter, 2010; Rodriguez-Saona et al., 2011). In this sense, the large-scale European efforts at protecting locally-grown, old fruit cultivars and studying ancestral cultivars in search of unique endemic germplasm in their centers of origin (e.g.,

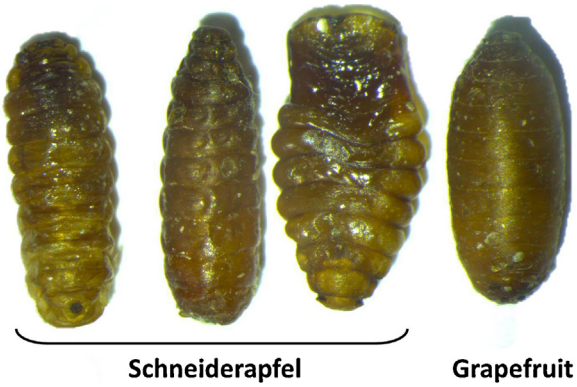

Fig. 4. Highly deformed pupae or hardened larvae, respectively, unable to fully complete metamorphosis.

Kazakhastan, Kyrgyzstan, and China [Sichuan, Chongqing]) should be considered of high strategic value (Kellerhals, 2009).

Augmenting the diversity of plant cultivars in agricultural fields can greatly reduce the need for extrinsic insect pest control measures, improve crop productivity (Tooker and Frank, 2012) and increase agroecosystem resilience in areas where climate change is already wreaking havoc (Mijatović et al., 2013). Here we present locally-grown Swiss and Mexican apple cultivars exhibiting various levels of phenolic compounds and/or resistance to a potentially invasive insect pest, as candidates for both breeding programs and mixed plantations aimed at enhancing the resilience of apple agroecosystems to pest invasions under climate change scenarios. The content of phenolic compounds did not influence egg laying propensity, suggesting that females were unable to detect the potentially deleterious compounds, so that lethal cultivars could act as population sinks for invading or expanding tropical species and within orchards, could be used for mass egg-trapping. Locally-grown cultivars in both Switzerland and Mexico thus represent a highly valuable asset to counteract the potentially devastating effects of invasive insect species in areas where global climate change is allowing pests to expand their range from tropical to temperate areas.

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