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Effects of mass rearing on life-history traits of an invasive fruit moth species, *Grapholita molesta* (Busck)

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Abstract

The oriental fruit moth, *Grapholita* (*Cydia*) *molesta* Busck (*Lepidoptera*: *Tortricidae*), is one of the most destructive and economically important pests of stone and pome fruits worldwide. Peach is considered to be the primary host of this moth, but apple and pear have become increasingly important as late season hosts in the past decades. In this study we used two *G. molesta* strains, one maintained in the laboratory for over 70-80 generations and the other for no more than 15, to study the effects of long-term rearing on some life-history traits. Larval development and adult performance were measured by growing the larvae on three main host plants (peach, apple and pear) in order to detect possible changes in host suitability. Results showed that long-term rearing did not significantly affect the selected life-history traits measured for the two oriental fruit moth strains, with the exception of larval survival. Furthermore, it was found that peach, apple and pear were similarly suitable as hosts for the two strains. Larval development, however, was faster on peach than on any other host. Thus, differences in host plant suitability across the three host plants seem to exist. These differences could explain to some extent, the distribution and performance of *G. molesta* populations in the field and the preference of oriental fruit moth for peach over apple and pear.

Zusammenfassung

Der Pfirsichwickler, *Grapholita* (*Cydia*) *molesta* Busck (*Lepidoptera*: *Tortricidae*), ist einer der wirtschaftlich bedeutendsten Schädlinge im Stein- und Kernobstbau weltweit. In den letzten Jahren wurde im späteren Verlauf der Jahreszeiten neben dem Hauptwirt Pfirsich auch Apfel und Birne als Hauptwirte immer bedeutender. In dieser Masterarbeit wurden die Auswirkungen von Laborzuchtpopulationen auf ontogenetische Entwicklungsmerkmale von *G. molesta* untersucht. Dazu wurde eine alte Zuchtpopulation - 70-80 Generationen – mit einer jungen Zuchtpopulation – 15 Generationen - verglichen. Raupen wurden an Früchten der drei Hauptwirte (Pfirsich, Apfel und Birne) herangezogen und auf Unterschiede in der Entwicklung untersucht. Die Ergebnisse zeigten, dass die beiden Laborzuchtpopulation keine signifikanten Auswirkungen auf die Entwicklung haben. Einzige Ausnahme war die Überlebensrate der Raupen. Weiters war die Raupenentwicklung signifikant kürzer auf Pfirsich als auf Apfel und Birne. Daher scheint es eine unterschiedliche Eignung als Wirtspflanze zwischen den drei Hauptwirten zu geben. Diese Unterschiede könnten bis zu einem bestimmten Grad die Verbreitung und das Verhalten von *G. molesta* Populationen im Feld und die Präferenz für Pfirsich gegenüber Apfel und Birne erklären.

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1. Introduction

1.1 The oriental fruit moth

1.1.1 Origin and distribution

The oriental fruit moth *Grapholita* (*Cydia*) *molesta* Busck (*Lepidoptera*: *Tortricidae*), also known as oriental peach moth, is one of the most destructive and economically damaging pests of stone and pome fruits worldwide. It is native to North-western China (Rotschild & Vickers, 1991), but in the last century it has expanded its distribution range to other parts of Asia including Japan (Hokkaido, Honshu, Shikoku, Kyushu), Korea, the Caucasus regions and the Middle East (Komai, 1999). The moth is also present in Australia (Northern Victoria and NSW), New Zeeland (North Island), Africa (Morocco, Mauritius Islands, South Africa), North America (From Canada to Florida, including the Atlantic Coast and Mississippi-Missouri Valley) and South America (Argentina, Uruguay, Chile and Southern Brazil) (Bellerose et al., 2007; Komai, 1999; Roehrich, 1961; Rotschild & Vickers, 1991; Tetsuya & Tatsuo, 2001; Timm et al., 2008). In Europe, the oriental fruit moth is present in Italy (Northern Italy, Sardinian Island, Sicilian Island), France, Portugal and Greece (Tremblay, 1990). Concomitantly with the present climate change scenario (IPCC, 2007), this moth has continued expanding its range, invading countries like Spain, Portugal-Azores, Albania, Montenegro, Macedonia, Romania, Serbia, Bosnien-Herzegovina, Bulgaria, Czech Republic, Slovenia, Slovakia, Hungary, Denmark, Lithuania, Russia, United Kingdom, Germany and Switzerland (Lopez-Vaamonde et al., 2010) (Figure 1).

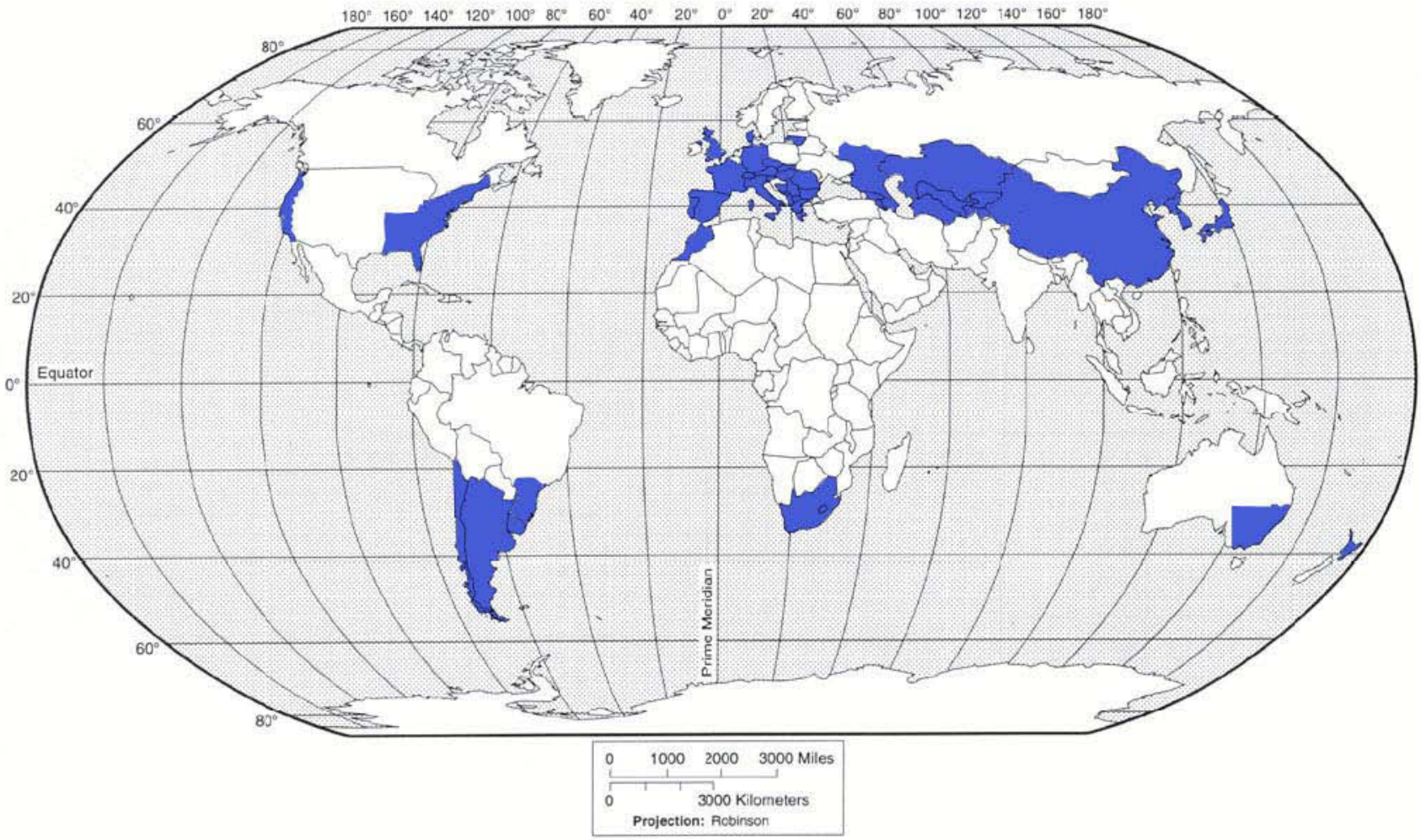

Figure 1. Worldwide distribution of *Grapholita molesta* according to literature reports from 1930-2010

1.1.2 Host range

The main host plants of the oriental fruit moth are species of the genus *Prunus* (*Rosaceace*, subfamily *Drupaceae*), including peaches, nectarines, plums and apricots (Tremblay, 1990). Among these plants, peach is considered to be the primary host (Rotschild & Vickers, 1991). Since host plants of the oriental fruit moth belong to the family *Rosaceae*, this moth is considered to be an oligophagous species. In the past decades, *G. molesta* has become an increasing problem not only in peach but also in apple and pear orchards worldwide (Borchert et al., 2004; Il'ichev et al., 2007). Host plant shifts of *G. molesta* from peach to apple and pear often occur late in the season for reasons still unknown (Natale et al., 1999), but differences in host plant suitability or availability might be one of such reasons (Myers et al., 2007).

1.1.3 Biology and life cycle

The oriental fruit moth is a multivoltine species (Rotschild & Vickers, 1991), thus, depending on the geographical region, several generations per year can develop in different host-plant tissues (Rotschild & Vickers, 1991) and crops (Myers et al., 2007; Natale et al., 2003). In Northern China, for instance, the first and second generations attack newly formed peach and apple shoots, the second and third generations attack peach shoots and fruits and the third to fifth generations attack apple and pear fruits (Zhao et al., 1989). Similarly, in eastern North America, the larvae feed on growing shoots of both peach and apple early in the season, whereas later in the season larvae feed primarily on fruits (Myers et al., 2006a; 2006c). In Europe, there are between four to five generations per year alternating between peaches and apples or pears. In Italy and France, however, it was recently reported that some *G. molesta* populations are able to reproduce throughout the whole year in pome fruits (e.g. apples and pears) alone. Given this ability to reproduce in several host plant species and in various types of host plant tissues, the development of an efficient monitoring technique for the oriental fruit moth adult populations represents an enormous challenge for insect pest managers (Pinero & Dorn, 2007).

The completion of development from egg to adult takes between 30 and 40 days under natural conditions (Dustan & Armstrong, 1930; Peterson & Haeussler, 1930; Rotschild & Vickers, 1991). Under laboratory conditions, however, this cycle might be shorted to only 20 to 25 days (N. Bellutti's personal observations). Developmental time is affected by environmental conditions particularly temperature. Eggs, larvae and pupae develop significantly faster at 30°C than at 22 and 25°C. Survival rates of eggs and larvae are also significantly higher at 30°C than at 33°C, whereas development precludes at 35°C (Notter-Hausmann & Dorn, 2010).

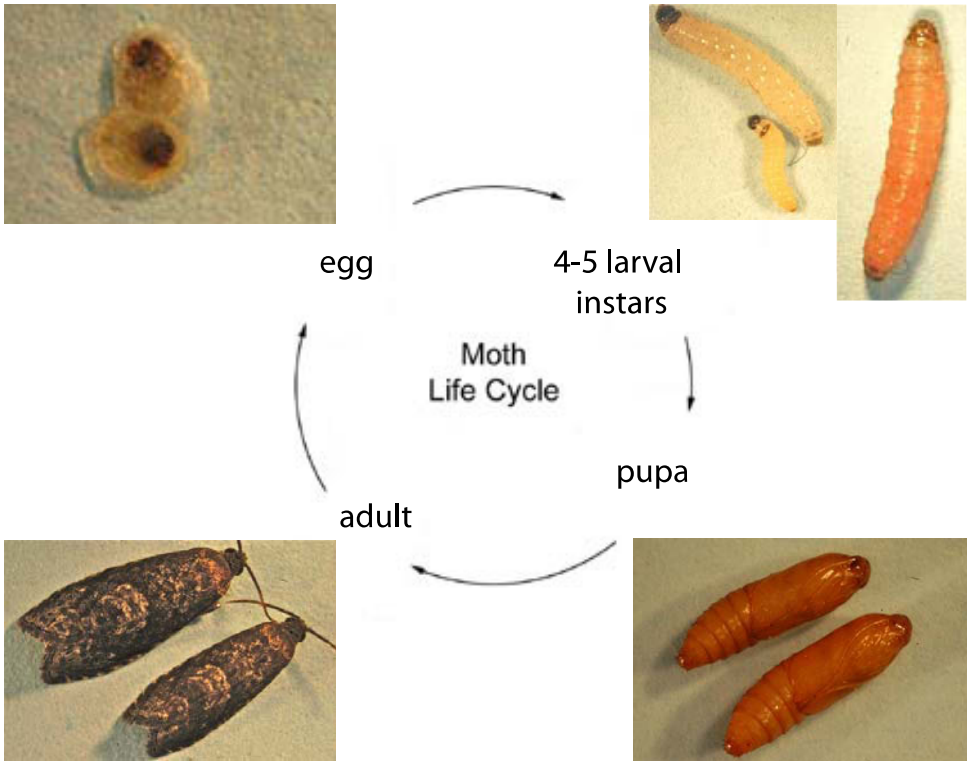

Figure 2. Life cycle of *Grapholita molesta*

The first moths emerge from late march to mid-May. In peach orchards, eggs are laid near young shoots, on the underside of leave and occasionally on young twigs (Figure 2). In apple and quince trees, the eggs are laid on the upper leave surface (Peterson & Haeussler, 1930; Smith & Summers, 1948). In pears, by contrast, eggs can be found on both sides of the leaves (Peterson & Haeussler, 1930). Eggs can also be laid on any type of smooth surface along newly formed peach and pear twigs (Peterson & Haeussler, 1930). Later in the season eggs are laid on the fruits.

Depending on the geographical region and the local climate, there might be 4-5 larval instars (Myers et al., 2007; Peterson & Haeussler, 1930). Under laboratory conditions, they generate 5 larval instars (N. Bellutti's personal observations). On shoots, the first instar larvae penetrate the leaves at their base, tunneling a 2 to 4 cm long gallery (Tremblay, 1990). On the fruits, by contrast, the larvae first tunnel into the fruits (Figure 3), and then feed from the flesh without injuring the seeds (Tremblay, 1990). The perforation of the plant tissue (either a shoot or a fruit) takes between 1.3 and 3 hours, after which the larva is no longer accessible to chemical treatment. This short time window of larval exposure limits the efficacy any chemical or biological control method against this species.

When larval development is completed, the late instars abandon the fruit and migrate to the lower parts of the tree in search of suitable hiding places to pupate (Peterson & Haeussler, 1930).

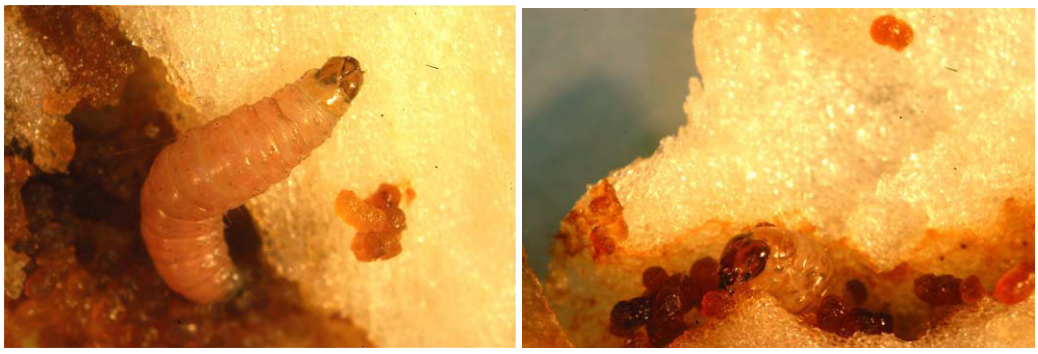

Figure 3. 5th instar *Grapholita molesta* larvae feeding on apple

1.1.4 Geographical range and damage

The presence of *G. molesta* in fruit orchards was first reported in Japan, then in Australia in 1909, in the American continent in 1916 and finally in Europe in 1920 (Rotschild & Vickers, 1991; Tremblay, 1990). Nowadays, *G. molesta* is present in most stone and pome fruits growing areas of the world (Figure 4) (Rotschild and Vickers, 1991).

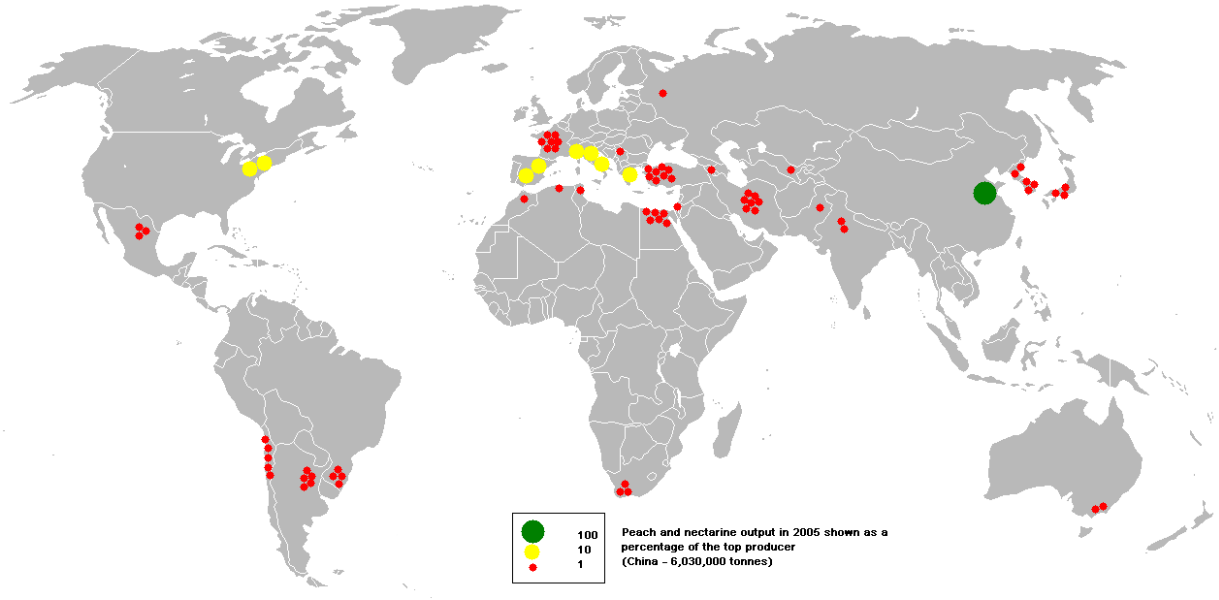

Figure 4. Global distribution of peach and nectarine output in 2005 as a percentage of the top producer China

Affected shoots wilt and might eventually die out and drop off the trees (Tremblay, 1990) (Figure 5). Damaged fruits, on the other hand, are characterized by the presence of gummy secretions and fungal growth, particularly of *Monilia fructigena,* by darkened tunnels inside the fruit (Figure 3 and 6) and by their early dropping off the trees (Tremblay, 1990). Many larvae can coexist in the same fruit, which can further increase the level of fruit damage.

Figure 5. Peach shoots damaged by *Grapholita molesta* larvae

Peach, the main host of the oriental fruit moth, is the third most produced fruit species, after apple and pear (FAOSTAT, 2009), in the world. The worldwide peach production estimated by FAO was of about 18 million tons in 2009 and involved 48 countries (FAOSTAT, 2009). The main producer is China with about 8.5 million tons, followed by the Mediterranean area (mainly Italy and Spain) and The United states of America (FAOSTAT, 2009). The secondary hosts, apple and pear, are cultivated in several countries and the yearly production in 2009 was around 72 tons for apple and 22 tons for pear (FAOSTAT, 2009). In Switzerland, apple is one of the most popular and consumed fruits. More than 61% of the 7000 ha of fruit cultivation are reserved for apples and the average national consumption rate is approximately 15.8 kg per capita (Obstverband, 2010). Worldwide, apple is regarded as high valuable commercial fruit and it is the second most important one in the international fruit trade market, after bananas.

Due to the low damage thresholds in fruit production (1-2%), and the increasing demands of zero pesticide residue levels on fruits, there is currently a strong demand of alternative and environmentally friendlier methods to control populations of *G. molesta* in the field.

Figure 6. Apple fruit damaged by *Grapholita molesta* larvae

1.1.5 Daily patterns of activity

Adult moths normally emerge during the early hours of the morning on sunny days, between 9-11am (Peterson & Haeussler, 1930; Smith & Summers, 1948), and fly at temperatures between 10-15 °C. The peak of daily activity is in the evening, around 2.5 h before the end of evening twilight, i.e. dusk (Natale et al., 2003). However, this time range can be shifted to earlier or later hours of the day (depending on the prevailing temperature conditions) across the season (Peterson & Haeussler, 1930; Smith & Summers, 1948). Mating takes place normally in the evenings. The first mating can occur as early as on the first day after adult emergence, but the first successful mating apparently takes place only during the second or third day after emergence. The maximum period of daily flight and oviposition seem to take place in the evening between 6 and 9pm (Dustan, 1964; Peterson & Haeussler, 1930). However, as the season progresses and the day length shorten, both flight and oviposition might be shifted to earlier hours of the day (Dustan & Armstrong, 1930; Smith & Summers, 1948).

1.1.6 Control methods

Current control measures against *G. molesta* are based on integrated pest management (IPM) strategies, combining chemical, biological and cultural approaches to achieve both economical and ecological pest control. For several decades, the most successful strategy to control this moth has been a combination of pesticide treatments and wide-area mating disruption. Mating disruption is based on males following a female sex pheromone trail to locate virgin females (Cook et al., 2007; Il'ichev et al., 1999). Dispensers in orchards thereby propagate a synthetic sex pheromone and the pheromone becomes ubiquitous. Subsequently, male moths are incapable of locating calling virgin females. Females remain virgin and reproduction is impeded. The population density and the mobility of mated females are important for the success of this technique (Carde & Minks, 1995). If population density is high, the probability of successful mate encounter is also high even under mating disruption treatment. Furthermore, if gravid females have a high flight capacity, they might migrate into new orchards, in which mating disruption is not practiced, and might oviposit there. This might undermine the effect of mating disruption. Indeed it has been shown that mated females of the oriental fruit moth are capable of inter-orchard flights (Hughes & Dorn, 2002). Apple and pear orchards near peach orchards are then endangered by immigration of gravid females late in the season. As mating disruption cannot control females, it is necessary to develop novel and efficient tools for monitoring and controlling *G. molesta* mated females. Semiochemical-based approaches have been suggested as potential useful tools in the case of this species (Natale et al., 1999).

1.2 Insect development and host plant suitability

Host plant location and acceptance are essential processes in the life history of herbivorous insects. Most of these insects use volatile and visual cues to find suitable hosts for mating and feeding, and in the case of gravid females, to lay the their eggs. The host plant chosen by gravid females must be suitable for insect larval growth and development. Plants species, however, differ in their degree of suitability for larval insect growth, particularly due to differences in nutritional levels and mechanical and chemical defenses (i.e. secondary metabolites). Thus, larval host plant directly affects both insect life-history traits and adult fitness (Gianoli et al., 2007). For many insects, particularly those that do not feed during the adult stage, adult fitness including body size, fecundity and longevity are strongly determined by resources obtained during the larval stage.

In the case of *G. molesta*, previous research indicated that development and reproduction is significantly affected by host plants, particularly in terms of oviposition preference (Myers et al., 2006a; 2006b), larval developmental rates and reproductive output (Myers et al., 2006c; 2007). In these studies, oriental fruit moth larvae reared on growing or excised apple fruits was found to have higher pupal mass, longer adult lifespan, oviposition period and fecundity compared to those reared on peach fruits. Larval survival was also found to differ between apple and peach tissues (i.e. shoots and fruits) and this difference varied according to the tissues' maturity levels. When immature fruits were tested, no differences in larval survival were found, however later in the season, larval development was faster in peach than in apple fruits. Earlier studies also found differences across peach and apple-reared insects in the laboratory, with those reared in apple developing slower than those reared in peaches (Peterson & Haeussler, 1930; Stearns & Neiswander, 1930). Despite the increasing importance of pear as a late season host for *G. molesta*, up to date only the study by Stearns and Neiswander (1930) has determined the effect of this host plant in larval feeding and development in relation to other hosts.

1.3 Effects of long-term rearing on insect colonies

The quality of laboratory-reared organisms is known to decrease over time. After introduction of a wild population from the field, the genetic variability of the base population ("founder effect") and its size tends to decrease as only those organisms that are better adapted to the rearing conditions (ie. artificial diet, rearing temperature, humidity and the limited space of the rearing cages) are selected (bottleneck effect). Thereafter a rearing 'strain' (i.e. a laboratory stock) that is well adapted to laboratory conditions is obtained, but both inbreeding depression and random genetic drift can further decrease the genetic variability of the new strain (Miyatake, 1998)(Figure 7). Both behavioral and physiological changes might occur in these organisms as a result of their adaptation to their particular rearing conditions, however very few studies have included this aspect into consideration as part of their research (Miyatake, 1998). For the melon fruit fly (*Bactrocera cucurbitae*), changes in different life-history traits were detected (Miyatake, 1998). Mass-reared flies showed a higher fecundity, fertility of eggs and frequency of oviposition and a larger number of mating attempts compared to wild flies. The mass-reared flies also showed also shorter life cycles compared to their wild counterparts.

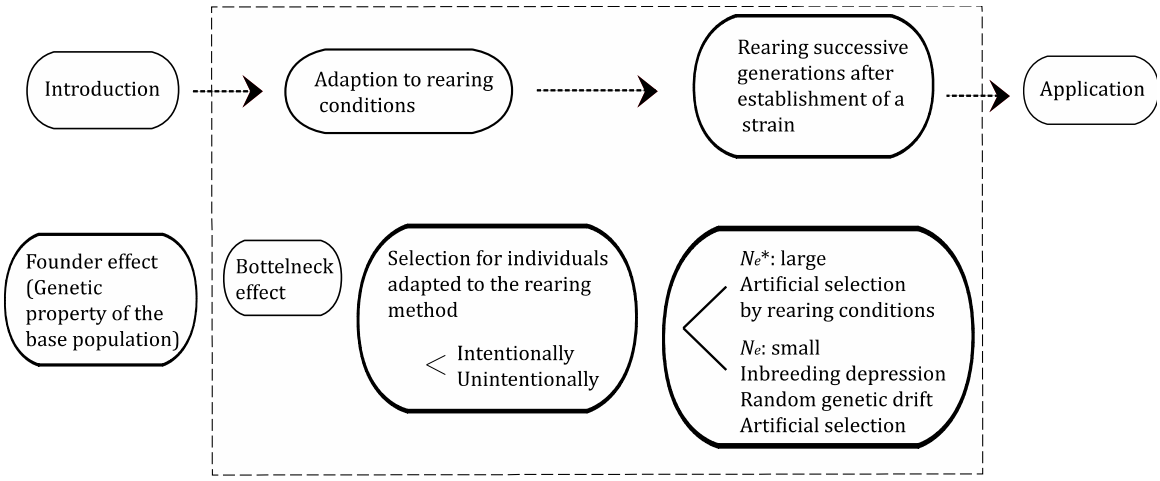

Figure 7. Steps during establishment of an insect rearing strain in relation to quantitative genetic aspects. *Effective population size. (Miyatake, 1998).

1.4 Aims of the study

In this study we used two *Grapholita molesta* strains, maintained in the laboratory for over 70-80 ('2007' strain) and for no more than 15 ('2009' strain) generations, as model organisms to study the effects of long-term rearing. Life-history traits on three field host plants (peach, apple and pear) were also measured in order to detect possible changes in host suitability for the two strains.

2. Material and Methods

2.1 Insects

Newly emerged *G. molesta* larvae used in this study were obtained from two different laboratory strains reared at the ETH Applied Entomology on an artificial diet (Huber, 1972) (Figure 8a). One strain originated from larvae collected in peach orchards in the Emilia-Romagna region (Italy 2007). The second strain was collected in the same region but in 2009. At the time of the experiments the former strain had been reared for over 70-80 generations in the laboratory and the latter for no more than 15 generations. The former strain will be referred to as the '2007' strain and the latter as the '2009' strain, from here onwards in the text.

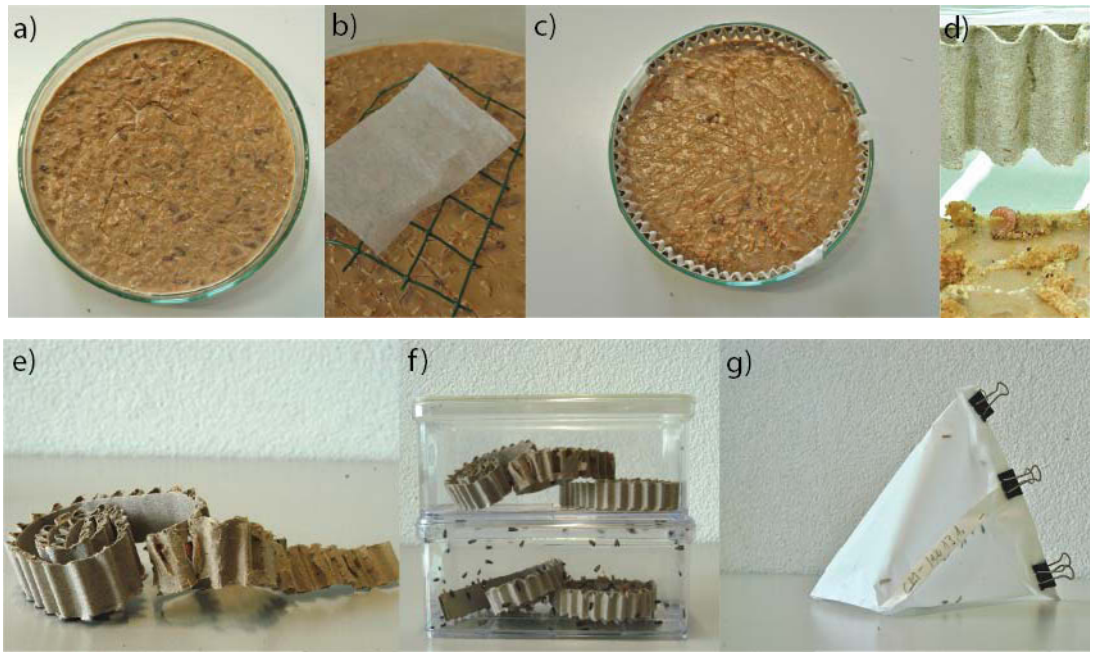

Figure 8. Laboratory rearing of *Grapholita molesta*. (a) Petri dish containing artificial medium. (b) Piece of egg paper (after disinfection) laid on artificial medium as to allow egg hatch. (c) Petri dish with attached cardboard (d) Cardboard with 5th instar larvae (e) Replaced cardboard with pupated larvae (f) Plastic container with cardboard and newly emerged adult moths (g) Baking paper cylinder for adult keeping and oviposition.

For maintenance of the two colonies, adults were kept in an insectary at 21°C, 60% RH and L:D 16:8h, in a baking paper cylinder (Figure 8g), allowing mating and egg laying on the walls. Moistened cotton was added to avoid dehydration. Every two to three days, remaining living adults were transferred to a new cylinder together with newly hatched individuals. Eggs laid on the cylinder were kept in a separate plastic bag provided with moist paper just before eclosion. Eggs were then disinfected by means of a short dip into a 10% hypochlorite solution and placed into glass Petri dishes (18cm diameter, 4cm height) (Figure 8b) containing an agar-based artificial diet (3mm height) (Huber, 1972) to allow egg hatching and subsequent larval growth. One week later, 2cm strips of corrugated cardboard, sterilized for 24h at 100°C, were attached to the rim of the Petri dishes to allow pupation of the last instar larvae (Figure 8c and d). The strips were than placed into new plastic containers (20cm x 10cm x 8cm) until adult emergence (Figure 8e and f). Newly emerged adult moths, males and females, were finally transferred to a new baking paper cylinder. Water was provided *ad libitum*.

2.2 Plant material

Ripening peaches, pears and apples fruits used in this study (Figure 9 and Table 1) originated from an organic farmer in the Canton of Ticino, Switzerland (46°23'43.73"N, 8°53'11.67"E) (Figure 10). Peach trees were of the cultivar Red Haven, pear trees of the cultivar Harrow Sweet and apple trees of the cultivar Resi Trees were treated only once, against fungi, in spring with cooper.

Table 1. Weight (mean ± SE) of the fruit material infested with first instar *G. molesta* larvae originated from two different laboratory strains.

Fruit species	Collection date	Weight (g)
Peach	03.08.10	86.03 ± 3.58
Apple	03.08.10	88.22 ± 3.15
Pear	03.08.10	87.18 ± 2.15

Figure 9. Peach (left), pear (middle) and apple (right) fruits used in this study

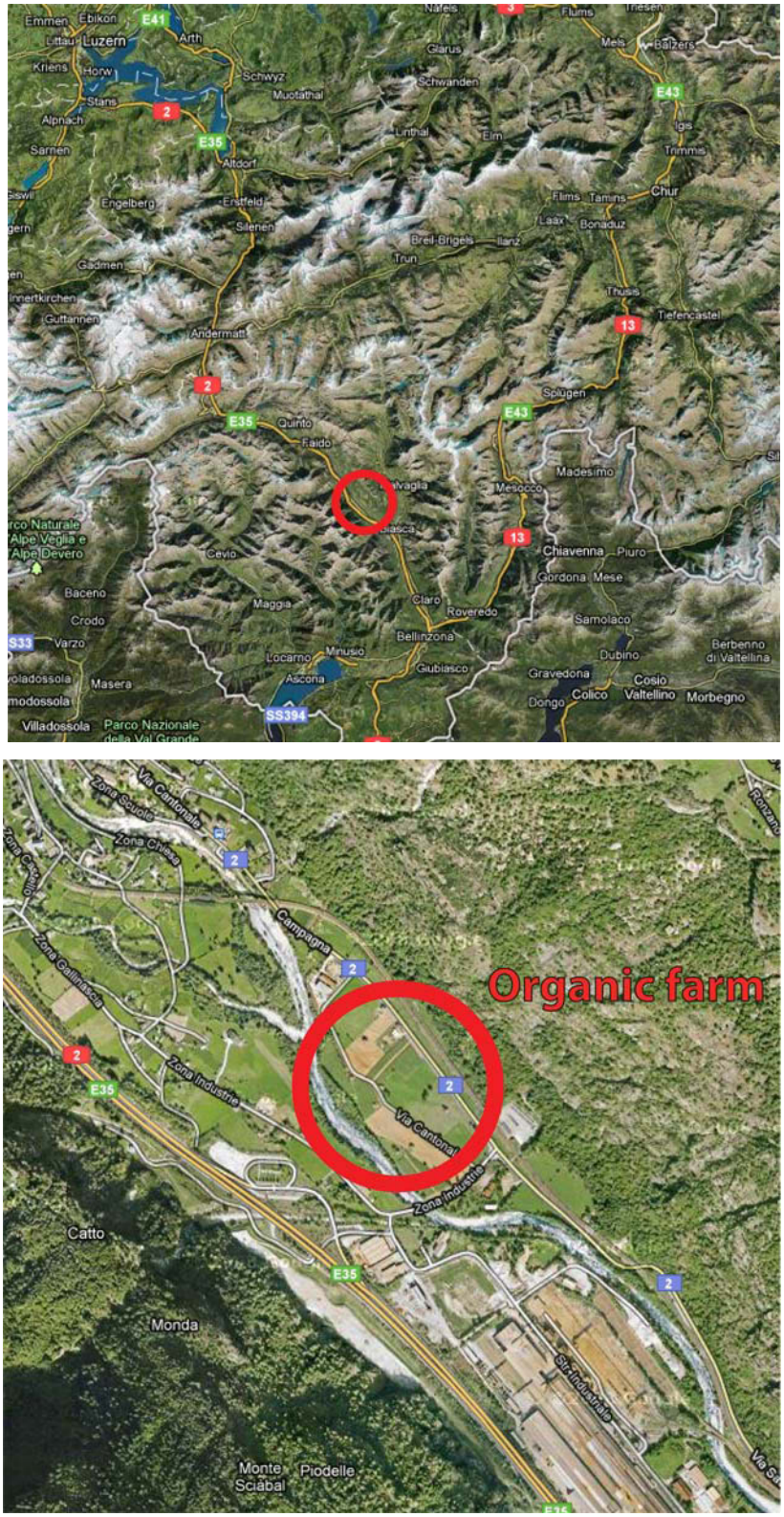

Figure 10. Location of the organic farm in the Canton of Ticino, Switzerland

2.3 Effects of larval food source on *G. molesta* life-history traits

To compare the performance of the two different *G. molesta* strains, newly emerged larvae from both strains were grown on four different food sources: either green peach, apple or pear fruits (Table 1) or agar-based artificial diet (Huber, 1972).

Eight fruits per plant type were infested with six newly emerged *G. molesta* larvae (n= six larvae/fruit) from the two strains (n= 48 larvae/strain). Simultaneously, eight plastic Petri dishes (14cm diameter, 2cm height) containing an agar-based artificial diet (2mm height) (Huber, 1972) were also infested with six first instar larvae from the two strains (n= 8 petri dishes/strain and 48 larvae/strain). Previous to infestation, fruits were immersed three times in deionisate water to remove any copper residue present on the fruit skin. Fruits were then air-dried.

Infested fruits were kept in plastic containers (20cm x 10cm x 8cm) (Figure 11a) provided with a glass ring (Figure 11b) as to allow the fruits to stand still and with paper towel at the bottom to provide a substrate for pupation. Plastic containers were kept in a controlled climate chamber (Conviron Ltd., Winnipeg, MB, Canada), with L:D 16:8, 60% RH and 24°C. Infested fruits were checked daily to monitor fruit health and larval emergence. Once fully developed larvae emerged from the fruit, they were allowed to build their cocoons on the paper. They were then withdrawn from the containers and the fresh weight and the sex of each newly formed pupa were recorded. Each pupa was placed into a separate plastic box (6cm x 4cm x 2cm), which was checked daily for adult emergence. Newly emerged adults were weighed, sexed and returned to each separate box, which was then provided with a wet cotton plug to avoid insect dehydration.

Newly emerged adult females were paired with males from the same line but from the stock cultures. The number of eggs laid by each female and the days until female death were recorded. Newly emerged males were not mated but kept in their individual boxes until death.

For each individual used in this study we calculated: (a) larval developmental time as the number of days between infestation date and emergence from the fruit, (b) adult longevity as the number of days between adult emergence date and death and (c) female fecundity as the number of eggs laid by females during their lifespan. For each strain and fruit type, both the percentage of larval and pupal survival was also calculated.

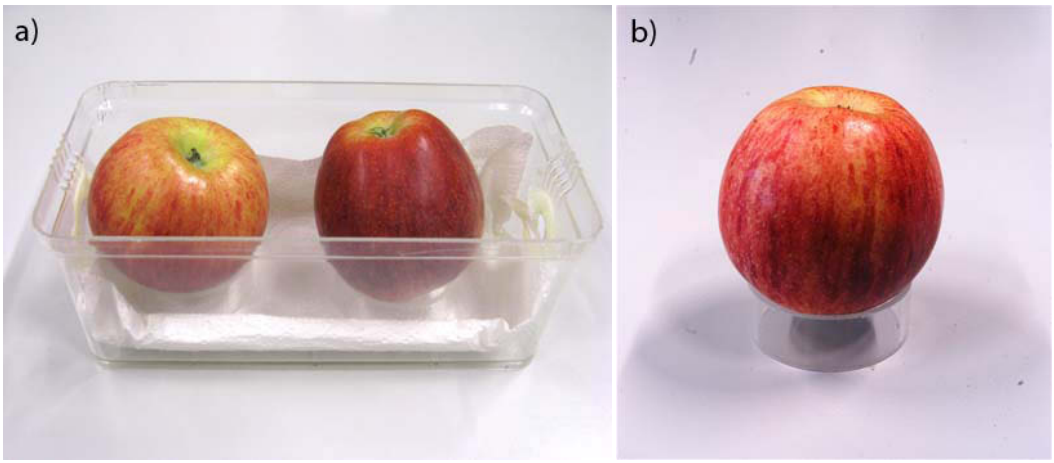

Figure 11. (a) Plastic container used to grow *Grapholita molesta* larvae on different fruits. In this case apple fruits were used. (b) All fruits used were placed on a glass ring to keep them still.

2.4 Statistical analyses

A multi-factorial analysis of variance (ANOVA) with sex, fruit type and strain as independent factors was conducted for each life-history trait parameter measured, namely larval developmental time, pupal weight, adult weight, adult longevity and female fecundity. When significantly differences within any of the factors were detected, means were separated by Tukey HSD tests with α set at 0.05. Prior to analysis, the distribution of the residuals was inspected to check for data normality. Levene's test was then used to check for homogeneity of variance. Since all data were normally distributed, no data transformation was conducted. To test for correlations between life-history trait parameters, pair-wise Pearson correlation analyses were also conducted. Percentage of surviving larvae and pupae were analyzed with a Z-test. Individuals that did not survive until the end of the experiment were excluded from the statistical analysis. All statistical analyses were performed using JMP version 8.0.2.

3. Results

Peach, apple, and pear fruits were similarly suitable as hosts for *G. molesta* individuals from both the '2007' and '2009' strains. However, some differences in the performance of the two strains on the different fruit types were detected, and these are described below.

Across all fruit types and strains, *G. molesta* male larvae developed faster than female larvae ($F = 8.19$, $df = 1$, $P < 0.01$, $N = 246$). However, female and male larvae from both strains developed significantly faster on peach fruit and artificial diet than on pear and apple fruits. Male and female larvae from the '2009' strain developed slightly faster, though not significantly so, on peach and apple fruits compared to the larvae from the '2007' strain and slightly slower, although not statistically different, than male and female larvae from the '2007' strain on both pear fruits and artificial diet (Figure 12).

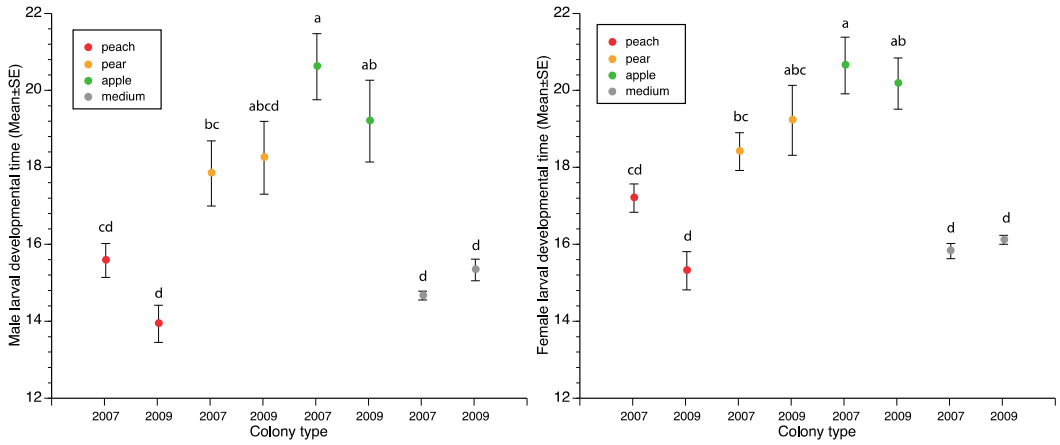

Figure 12. Mean larval developmental time of male (left) and female (right) *Grapholita molesta* individuals from two different strains, '2007' and '2009', developed on three different fruit types and artificial diet as a control. *N* = 74 males and 69 females for the '2007' strain and *N* = 46 males and 57 females for the '2009' strain. Means followed by the same letter are not significantly different (Tukey HSD test; *P* < 0.05).

Mean weight of newly formed pupae did not differ statistically across strains and larval hosts, neither for male nor female individuals. Female pupae were, however, heavier (F = 162.0, df = 1, P< 0.0001, N = 246) than male pupae. Lower female weight values were achieved when the '2009' strain was grown on peach fruits and when the '2007' strain was grown on apples. The highest mean female pupal weight was recorded when the '2009' strain was grown on apple fruits (Figure 13).

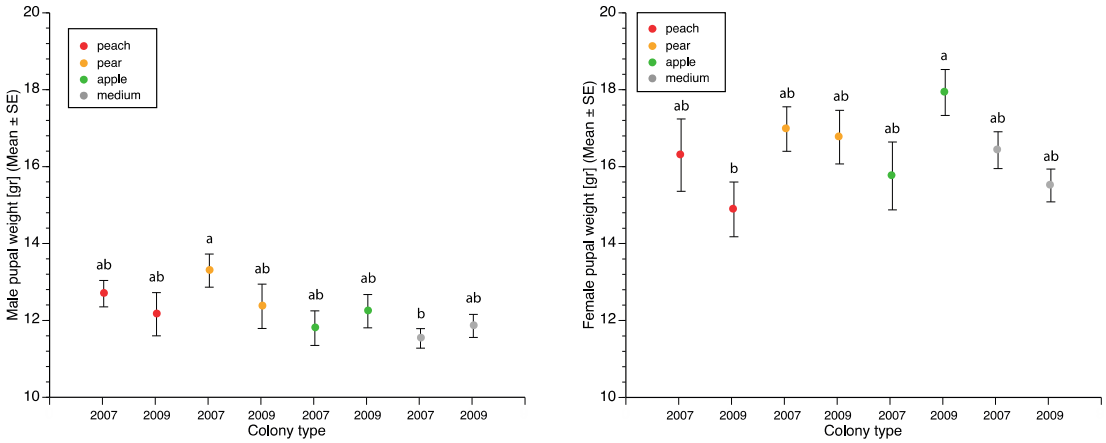

Figure 13. Mean pupal weight of male (left) and female (right) *Grapholita molesta* individuals from two different strains, '2007' and '2009', developed on three different fruit types and artificial diet as a control. *N* = 74 males and 69 females for the '2007' strain and *N* = 46 males and 57 females for the '2009' strain. Means followed by the same letter are not significantly different (Tukey HSD test; *P* < 0.05).

Mean adult weight at emergence was positively correlated with fresh pupal weight for females and males emerging from both strains (Pearson correlation analysis: '2007' strain: r = 0.94, N = 69 for female and r = 0.83, N = 74 for male; '2009' strain: r = 0.94, N = 57 for female and r = 0.80, N = 46 for male). Thus, for pupal weight, higher values were recorded for females compared to males (F = 213.09, df = 1, P < 0.0001, N = 246), but no significant differences were detected across strains (F = 0.314, df = 1, P = 0.575, N = 246) or larval hosts (F = 1.085, df = 3, P = 0.356, N = 246) (Figure 14).

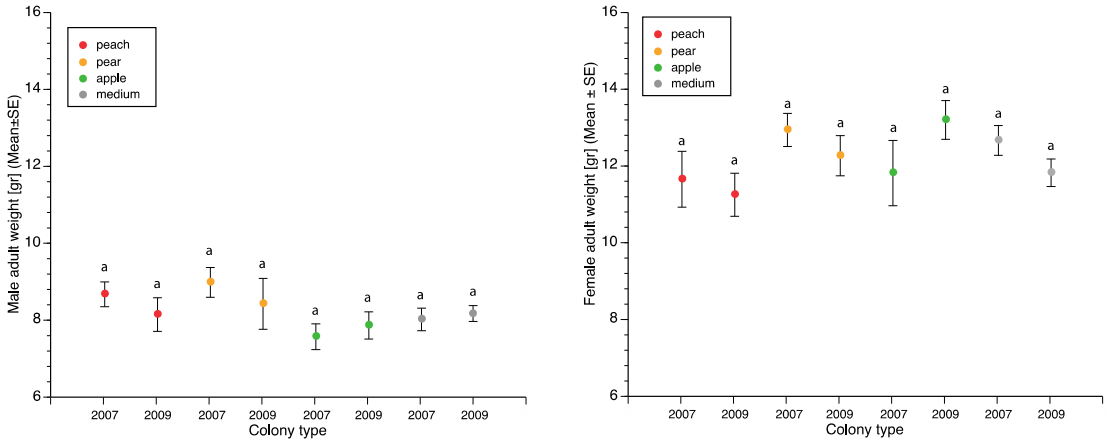

Figure 14. Mean adult weight of male (left) and female (right) *Grapholita molesta* individuals from two different strains, '2007' and '2009', developed on three different fruit types and artificial diet as a control. *N* = 74 males and 69 females for the '2007' strain and *N* = 46 males and 57 females for the '2009' strain. Means followed by the same letter are not significantly different (Tukey HSD test; *P* < 0.05).

Mean adult longevity did not differ significantly across sexes (F = 1.698, df = 1, P = 0.193, N = 246), strains (F = 2.508, df = 1, P = 0.114, N = 246), or larval hosts, with the exception of those adults from the '2007' strain reared on the artificial diet which lived significantly longer than all remaining adults (Figure 15).

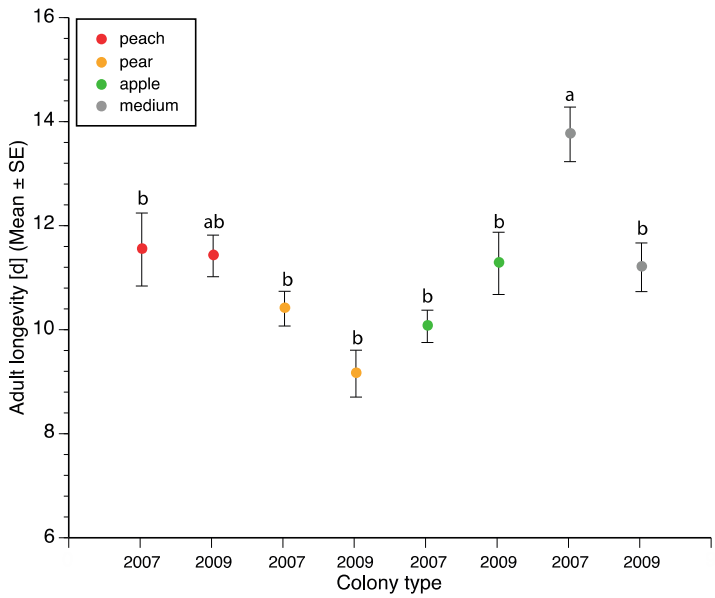

Figure 15. Mean adult longevity *Grapholita molesta* individuals from two different strains, '2007' and '2009', developed on three different fruit types and artificial diet as a control. *N* = 143 for the '2007' strain and *N* = 103 for the '2009' strain . Means with the same letter are not significantly different (Tukey HSD test; *P* < 0.05).

Mean female fecundity values varied, though not significantly so, across strains and larval hosts. Females from both strains laid slightly more eggs when grown on artificial diet and on peach fruits compared to when grown on pear and on apples. When grown on pears, however, females from the '2007' strain laid statistically more eggs than those from the '2009' strain and the opposite was true when females where grown on apples (Figure 16).

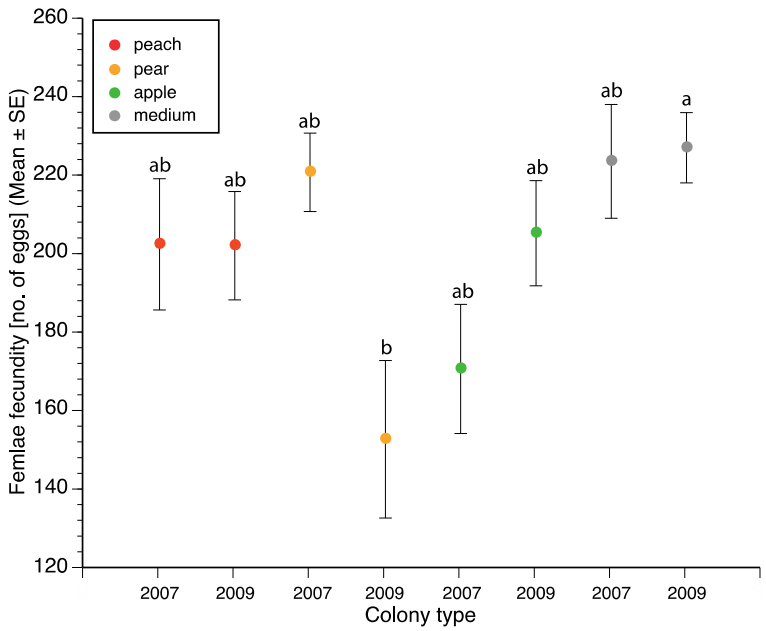

Figure 16. Mean fecundity of mated female *Grapholita molesta* individuals from two different strains, '2007' and '2009', developed on three different fruit types and artificial diet as a control. *N* = 143 for the '2007' strain and *N* = 103 for the '2009' strain. Means with the same letter are not significantly different (Tukey HSD test; *P* < 0.05).

Mean fecundity values were positively correlated with pupal weight values for *G. molesta* females from the '2007' strain grown on the three different fruit types (Pearson correlation analysis: $r = 0.92$, $N = 15$, for peach; $r = 0.86$, $N = 21$, for pear and $r = 0.86$, $N = 17$, for apple), but not when grown on artificial diet ($r = 0.28$, $N = 16$). This correlation persists for females from the '2009' strain grown on peach fruits, pear fruits, and on artificial diet (Pearson correlation analysis: $r = 0.79$, $N = 15$ for peach, $r = 0.81$, $N = 9$ for pear and $r = 0.81$, $N = 17$ for artificial diet), but not on apple fruits ($r = 0.14$, $N = 16$).

The percentage of larval individuals surviving (Figure 17) on the different hosts differed significantly across the two strains, but not across sexes. Individuals from both strains survived longer, although not significantly so, when grown on the artificial diet compared to any fruit type. Furthermore, individuals from the '2007' strain survived longer than those from the '2009' strain, with values being significantly different when the two strains were grown on pears (Table 2). Percentage of surviving pupae, on the other hand, was not significantly different across the different larval hosts or across the two strains.

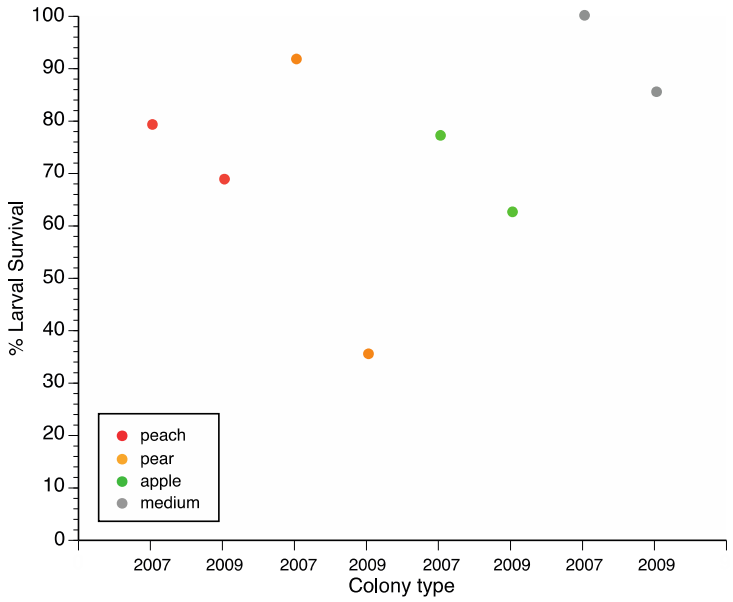

Figure 17. Percentage of surviving *Grapholita molesta* larvae from two different strains, '2007' and '2009', developed on three different fruit types and artificial diet as a control. *N* = 168 for the '2007' strain and *N* = 121 for the '2009' strain of a total *N* = 384

Table 2. Surviving differences of *Grapholita molesta* larvae from two different strains, '2007' and '2009', developed on three different fruit types and artificial diet as a control. (z-test; α < 0.05)

		2007				2009			
		peach	**pear**	**apple**	**medium**	**peach**	**pear**	**apple**	**medium**
2007	**peach**	-	P= 0.357^{ns}	P= 0.872^{ns}	P= 0.116^{ns}	P= 0.364^{ns}	P< .0001***	P= 0.152^{ns}	P= 0.116^{ns}
	pear	P= 0.357^{ns}	-	P= 0.280^{ns}	P= 0.514^{ns}	P= 0.068^{ns}	P< .0001***	P= 0.019*	P= 0.651^{ns}
	apple	P= 0.872^{ns}	P= 0.280^{ns}	-	P= 0.083^{ns}	P= 0.454^{ns}	P< .0001***	P= 0.203^{ns}	P= 0.529^{ns}
	medium	P= 0.116^{ns}	P= 0.514^{ns}	P= 0.083^{ns}	-	P= 0.013*	P< .0001***	P= 0.002**	P= 0.270^{ns}
2009	**peach**	P= 0.364^{ns}	P< .0001***	P= 0.152^{ns}	P= 0.116^{ns}	-	P= 0.001**	P= 0.598^{ns}	P= 0.169^{ns}
	pear	P= 0.068^{ns}	P< .0001***	P= 0.019*	P= 0.651^{ns}	P= 0.001**	-	P= 0.006**	P< .0001***
	apple	P= 0.454^{ns}	P< .0001***	P= 0.203^{ns}	P= 0.529^{ns}	P= 0.598^{ns}	P= 0.006**	-	P= 0.057^{ns}
	medium	P= 0.013*	P< .0001***	P= 0.002**	P= 0.270^{ns}	P= 0.169^{ns}	P< .0001***	P= 0.057^{ns}	-

4. Discussion

In this study we used two *Grapholita molesta* strains, maintained in the laboratory for over 70-80 ('2007' strain) and for no more than 15 ('2009' strain) generations, as model organisms to study the effects of long-term rearing. Life-history traits on three field host plants (peach, apple and pear) were also measured in order to detect possible changes in host suitability for the two strains.

Effects of long-term rearing on *G. molesta* life-history traits

Long-term rearing did not affect significantly the selected life-history traits measured for the two *G. molesta* strains used in our study. Indeed, significant differences across the two strains were only detected with regards to percentage larval survival, with larvae from the '2007' strain, surviving better on all food types than those from the '2009' strain. That the '2007' strain survived better than the '2009' one most likely indicates a better degree of 'adaptation' of the former strain to our rearing conditions compared to the latter. Earlier studies detected slight differences between *Grapholita molesta* field and laboratory reared lines (on picked green peaches and apples), in, particularly due to prevailing cold conditions in the field which tended to delay development (Peterson & Haeussler, 1930). Although it would have been ideal to compare the effect of long-term rearing on field vs laboratory *G. molesta* strains, the quarantine status of this moth in Switzerland, where this study was conducted, did not allowed us to conduct such comparison.

In other insect species, long-term rearing seems to affect in life history traits that compromise both the production efficiency in the laboratory and the quality of mass-reared insects. For instance, in the closely related species, *C. pomonella*, a trade-off between mobility and fitness across two strains with contrasting mobility was documented. Female moths of the less mobile strain were significantly larger, laid more eggs during their lifespan, and both male and females of this strain lived longer, when compared to the other strain (Gu et al., 2006). In melon flies, *Bactrocera cucurbitae*, long-time rearing increased longevity, which was associated with later

age of reproduction, developmental time and lpre-adult survival rate (Miyatake, 1998). Mass rearing effects on life-history traits and inbreeding depression were also detected in the sweetpotato weevil, *Cylas formicarius elegantulus* (Kuriwada et al., 2010). The mass-reared strain had more progeny and a shorter developmental period compared to the wild strain. In honeybees, inbred worker bees led to depression in the colony performance through reduced honey and wax production. Swarming behavior and calmness during examination showed also significant differences according to the inbreeding level of queens and workers (Bienefeld et al., 1989).

Although in our study we did not detect any significant effect of long-term rearing on *G. molesta* life-history traits, behavioral traits which are closely related to fitness (e.g. olfactory orientation and mating behavior) could suffer from a greater inbreeding depression than morphological traits (DeRose & Roff, 1999). Indeed, we found that *G. molesta* females from the '2007' strain showed significantly less olfactory discrimination than those from the '2009' strain (Bellutti et al., 2011). Thus, we suggest that all future studies with *G. molesta* should use strains recently collected from the field, or reared for as few generations in the laboratory as possible, to ensure sufficient amount of genetic diversity (Mackauer, 1972) in the strain and thus maintain a high insect quality. This will reduce the chances of obtaining underestimated results that could delay or jeopardize our research expectations regarding this moth species.

Host plant suitability

Larval host suitability is known to affect life-history traits related to fitness in many insect species (Moreau et al., 2007; Myers et al., 2006a; 2006b; 2006c; 2007; Neiswander & Stearns, 1930; Peterson & Haeussler, 1930; Thiery & Moreau, 2005). In our experiments, larval development was the only life history trait significantly affected by larval host type. *Grapholita molesta* larvae from the two different strains developed faster on an artificial diet or on peach fruits, the insects' primary host, than on pear or apple fruits, two of the most important secondary hosts of this insect moth in the field. Studies conducted earlier in the past century, with *G. molesta* larvae

grown on green apples and peaches, showed related results (Peterson & Haeussler, 1930). Larval development was different across peach- and apple-reared insects, with those reared on peaches developing faster (ca. 12 days) than those reared on apple (ca. 15 days). Similar results were also recently documented for *G. molesta* on peach and apple shoots and fruits, with larvae developing faster on peach than on apple, both on growing terminal shoots as well as on fruits .(Myers et al., 2007)Additionally, Neiswander and Stearns (1930) showed that larvae developed fastest on peach compared to plums, apples, pears and quinces and that the average feeding period was lower in peaches (13.8 days) compared to apples (16.7 days) and pears (16.9 days).

Host plant shifts of *G. molesta* from peach to apple and pear often occur late in the season for reasons still unknown (Natale et al., 1999), and differences in host plant suitability or availability might be one of such reasons (Myers et al., 2007). Our results seem to support the former assumption. Differences in host plant suitability across the three host plants could affect to some extent, the distribution and performance of *G. molesta* populations in the field and could be a driving force behind the preference of *G. molesta* for peach over apple and pear.

Interestingly, larval host type did not affect other life-history traits, including body weight, adult longevity, female fecundity and survival rates. Since adults of *G. molesta* were not fed during adulthood in our experiments, the acquisition of resources during the larval stage should have directly affected these adult fitness parameters. In contrast to our results, a generally higher survival of *G. molesta* larvae growing on peach compared to apple was documented previously in studies conducted in the USA (Myers et al., 2006c). These results were however affected by the fruit maturity level tested. In our study we only used green fruits for larval growth, as they are those more prone to be infested by gravid oriental *G. molesta* females in the field. Different larval hosts seem to differently affect other insect species (Barros et al., 2010; Diamond & Kingsolver, 2011). For instance, the closely related grapevine moth *Lobesia botrana* seems to *be* affected by larval diet. When *L. botrana* larvae were grown on alternative hosts (*Daphne gnidium, Olea europea, Tanacetum vulgare*),

larval developmental time and mortality were reduced, while pupal weight, growth rate, female fecundity and longevity, egg laying period and mating success were increased (Thiery & Moreau, 2005). Larval development time, egg size and egg hatchability were also significantly different when larvae were fed on particular grape cultivars (Moreau et al., 2007). Although female fecundity did not differ among varieties, females fed on some varieties produced eggs of different sizes and fertility levels (Moreau et al., 2006a). Larval food type can also affect sexual size dimorphism and adult fitness through host associated factors (Gianoli et al., 2007). In our experiments we found sexual dimorphisms in pupal and adult weight, with values being significantly higher for female individuals compared to male ones. Larval developmental time also differed significantly between males and females, with females emerging later than males. This phenomenon is called "protandric emergence" and is common across the Lepidoptera (Moreau et al., 2006b).

Recent outbreaks of *G. molesta* populations in apple and pear growing areas occurring in recent years have been attributed to host-driven processes (Myers et al., 2006b). Pear has only recently received some attention as a potential host for this moth, and our results seem to suggest that at least in terms of larval development and subsequent adult performance, this plant is as suitable host for *G. molesta* as apple and only slightly less suitable host compared to peach. In some cases host-associated races have been identified among major related tortricid pests, such as the codling moth, *Cydia pomonella* (Chen & Dorn, 2010; Piskorski et al., 2010) and the fruit tree leafroller, *Archips argyrospila* (Myers et al., 2006b). Development of *G. molesta* larvae seems to be affected by host plants, as shown in our experiments. However, unlike *C. pomonella,* evidence so far gathered seem to suggest that *G. molesta* forms no genetically distinct races but rather "ectomorphs", where different populations are affected differently by different host plants (Myers et al., 2006a). Further studies testing the mating status of *G. molesta* field populations collected on different host plants and different geographical regions could provide empirical evidence that will allow checking the validity of this statement.

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Figures

Figure 4.	http://en.wikipedia.org/wiki/File:2005peach_and_nectarine.PNG
Figure 5.	http://www.invasive.org/browse/detail.cfm?imgnum=1234036 http://entweb.clemson.edu/cuentres/cesheets/fruit/ce210.jpg
Figure 9.	http://www.baumschule-horstmann.de/shop/exec/product/ 65/6722/Pfirsich-Red-Haven.html http://www.artus-group.de/bilddownload_birnen.html http://www.saengerhof.de/fileadmin/templates/shop.php?wg=21
Figure 10	htp://maps.google.ch/map