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Is the alpine divide becoming more permeable to biological invasions? – Insights on the invasion and establishment of the Walnut Husk Fly, *Rhagoletis completa* (Diptera: Tephritidae) in Switzerland

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Abstract

The Walnut Husk Fly, *Rhagoletis completa* Cresson (Diptera: Tephritidae), is native to North America (Midwestern US and north-eastern Mexico) and has invaded several European countries in the past decades by likely crossing the alpine divide separating most parts of Switzerland from Italy. Here, we determined its current distribution in Switzerland by sampling walnuts (*Juglans regia* L.) in ecologically and climatically distinct regions along potential invasion corridors. *R. completa* was found to be firmly established in most low altitude areas of Switzerland where walnuts thrive, but notably not a single parasitoid was recovered from any of the samples. Infested fruit was recovered in 42 of the 71 localities that were surveyed, with mean fruit infestation rate varying greatly among sites. The incidence of *R. completa* in Switzerland is closely related to meteorological mean spring temperature patterns influencing growing season length, but not to winter temperatures, reflecting survival potential during hibernation. Importantly, areas in which the fly is absent correspond with localities where the mean spring temperatures fall below 7°C. Historical data records show that the natural cold barrier around the Alpine divide in the central Swiss Alps corresponding to such minimal temperatures has shrunk significantly from a width of more than 40 km before 1990 to around 20 km after 2000. We hypothesize on possible invasion/expansion routes along alpine valleys, dwell on distribution patterns in relation to climate, and outline future research needs as the incursion of *R. completa* into Switzerland; and, more recently, other European countries, such as Germany, Austria, France and Slovenia, represent an example of alien species that settle first in the Mediterranean Basin and from there become invasive by crossing the Alps.

Keywords: *Rhagoletis completa*, Tephritidae, invasive alien species, invasion ecology, Switzerland

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Introduction

Alien insect species have become an increasing concern worldwide due to their significant ecological and economic impact (Mack *et al.*, 2000; Mooney & Hobbs, 2000; Pimentel *et al.*, 2000; Clarke *et al.*, 2005; Roll *et al.*, 2007; Kenis *et al.*, 2009). For example, in North America alone, invasive species (insects and other organisms) have been estimated to cause damage and losses of ca. US \$137 billion per year (Pimentel *et al.*, 2000). According to Kenis *et al.* (2009), the majority of studies on the ecological effect of invasive insect species have been carried out in the past eight years, with two thirds of them occurring in North America and only about 5% in Europe. Invasions of herbivorous arthropods in North America reflect historical patterns of movement, with the vast majority of introduced species in Canada being of Palearctic origin, particularly from Europe (Langor *et al.*, 2009). Nevertheless, recent increases in trade with China have been accompanied by matching increases of invasive species of such origin (Langor *et al.*, 2009). Based on the latter, it is expected that augmented trade from North America to Europe will also be accompanied by new biological invasions. Data by Kenis (2006) shows that in Switzerland there are over 300 established alien insect species, with numbers likely to increase as a result of recent liberalization of human movement within the Schengen Territory and expanding trade with commercial partners worldwide.

The speed at which invasions occur has been significantly increased in recent times as a result of mainly two factors: (i) expanding international trade and tourism (Mumford, 2002; Work *et al.*, 2005; Westphal *et al.*, 2008), and (ii) global warming (Dukes & Mooney, 1999; Ricciardi, 2007). Global warming is also opening new pathways for alien organisms, as, for example, formerly impenetrable mountain passes gradually become milder, allowing the survival of adults that are wind carried (Chapman *et al.*, 2004; Torrez-Diaz *et al.*, 2007). Also, many invasive species, establishing transient populations that would normally vanish during cold winter months, now survive as a result of winter or, more importantly, spring months that are becoming milder (Cannon, 1998; Rosati & Van Laerhoven, 2007).

Among the most worrisome groups of invasive insects, fruit flies (Diptera: Tephritidae) stand out, as their invasive capacity impacts society along several axes. (i) On an ecological level, invasive species, particularly highly polyphagous ones, can displace native species or compete for essential resources with them (Duyck *et al.*, 2004). (ii) On an economic level, severe economic damage is inflicted as infested fruit and vegetables become unmarketable and inedible. In addition, the presence of invasive species severely restricts trade (Aluja & Mangan, 2008). (iii) On a social level, invasive species such as the Medfly, *Ceratitis capitata* (Wiedemann) or *Bactrocera invadens* Drew, Tsuruta & White can reap havoc, as wide-ranging eradication efforts can cause the appearance of secondary pests as natural enemy populations dwindle or entire local economies are sent into disarray because formerly stable trade commodities suddenly become scarce or cannot be exported anymore (Mumford, 2002; Drew *et al.*, 2005).

Here, we dwell on the relatively recent invasion into Switzerland of a fruit fly belonging to the genus *Rhagoletis* Loew native to North America (*R. completa* Cresson), detailing its current distribution within the country, analyzing some biotic and abiotic factors that explain its establishment and rapid expansion and offering hypotheses as to possible

invasion routes. Flies within the genus *Rhagoletis* are distributed in North and South America, all of Europe (Austria, Bulgaria, France, Germany, Greece, Italy, Netherlands, Norway, Poland, Portugal, Spain, Sweden, Switzerland, Turkey (EPPO/CABI, 1996)) and parts of Eurasia

In Switzerland, only *Rhagoletis cerasi* is endemic; but, in the past two to three decades, *Rhagoletis completa* and *Rhagoletis cingulata* (Loew) have invaded the country, most likely from Italy where the two species were first reported for Europe (Duso, 1991; Norrbom, 2004). *R. completa* was initially found near Venice and in the Friuli region (Duso, 1991), and its presence in Italy was shortly thereafter confirmed in Milano, Pavia, Novara, Varese and Sondrio (Ciampolini & Trematerra, 1992). The first formal reports of the presence of *R. completa* and *R. cingulata* (originally reported as *R. indifferens*) in Switzerland originate from trap captures in the Ticino region (Merz, 1991; Mani *et al.*, 1994; Lampe *et al.*, 2005), indicating that these flies possibly invaded the country in the mid- to late 1980s. As is the case with other non-European species of Tephritidae, *R. completa* was regulated as a quarantine pest in Switzerland and presently still possesses this status (Swiss Federal Council, 2001). As noted above, *R. indifferens* Curran was also reported in Switzerland (Merz, 1991; Mani *et al.*, 1994), but B. Merz later acknowledged that the specimens had been misidentified and were in fact *R. cingulata* (Merz & Niehuis, 2001). So, there is no formal evidence that *R. indifferens* is or was ever present in Switzerland or anywhere else in Europe.

Rhagoletis completa belongs to the *suavis* species group where it has been placed together with *R. boycei* Cresson, *R. suavis* (Loew), *R. juglandis* Cresson, *R. zoqui* Bush and *R. ramosae* Hernández-Ortiz (Bush, 1966; Hernández-Ortiz, 1985; Smith & Bush, 2000). It is native to Midwestern USA and north-eastern Mexico (Bush, 1966; Smith & Bush, 2000; Chen *et al.*, 2006) and was described from specimens collected in the USA in the late 1920s (Cresson, 1929). In the USA, it was originally restricted to the Midwestern part of the country, but between 1922 and 1925 it was reported in California (Boyce, 1934). On the west coast, the fly now ranges from southern California as far north as Washington State (Chen *et al.*, 2006). In Mexico, it is restricted to the north east (Smith & Bush, 2000) particularly the states of Coahuila, Nuevo León, and Tamaulipas (J. Rull, unpublished data). The known hosts of *R. completa* are *Juglans nigra* L., *J. microcarpa* Ber., *J. californica* S. Watson, *J. hirsuta* Manning, *J. hindsii*, Jepson ex R.E. Smith, *J. major* (Torr.) A. Heller and *J. regia* L. (Bush, 1966; Smith & Bush, 2000). In addition to infesting walnuts, *R. completa* has been reported infesting peaches, *Prunus persica* L. (Boyce, 1934), although this host affiliation is rare (Smith & Bush, 2000). The few additional details on its biology can be summarized as follows: it goes through obligate diapause, is univoltine, is considered oligophagous, and is attacked by very few parasitoids (Boyce, 1934; Legner & Goeden, 1987; Kasana & AliNiasee, 1995, 1996; Ovruski *et al.*, 2007). European populations in Italy exhibit one generation a year, with adult emergence spanning from early July to the second half of August. Oviposition occurs from late July to early September, with peaks between the 5th and 18th of August. First instar larvae have been recorded since early August, and mature larvae leave husks from late August onwards to pupate in the soil (Duso & Dal Lago, 2006).

Economic damage is caused by *R. completa* larvae, especially when the infestation occurs in an early stage of walnut development from July until mid-August. Damage has

been reported to occur on 74–91% of fruit in untreated orchards, with fruit not developing fully and producing small nuts or on occasion shrivelled, mouldy kernels (Olhendorf, 2000; Duso & Dal Lago, 2006). Furthermore, there is the suspicion that heavily infested fruit facilitates the penetration of pathogens into the edible nut, such as the fungus *Marssonina juglandis* (Lib.) Magnus and particularly the bacteria *Xanthomonas campestris* pv. *juglandis* (Pierce (Dye)), causing the actual nut (kernel) to shrink and lose weight and also to rot, producing considerable (sometimes total) yield loss as a consequence of mould growth and malformation of the kernel (Hislop & Allen, 1983; Coates, 2005). Despite the fact that feeding activity of *R. completa* larvae in late infestations usually does not damage the commercially valuable kernel, it does interfere with the natural separation of the pulp from the nut shell, and this can render commercialization cumbersome or impractical. Black stains have to be removed from the nut skin with high water pressure or nuts must be bleached as consumers refuse or hesitate to buy stained nuts (Hislop & Allen, 1983; Olhendorf, 2000).

Considering the fact that the history of *R. completa* in Europe and particularly Switzerland is fairly recent and that, since it was first reported in Italy by Duso (1991) and Ciampolini & Trematerra (1992), its presence has been confirmed in other European countries such as Slovenia (Seljak, 1999), Austria (C. Lethmayer, personal communication), Germany (EPPO, 2004) and France (EPPO, 2008; Bouvet, 2009), we decided to investigate the current distribution of *R. completa* in Switzerland, as possibly global warming was generating suitable conditions for its expansion through the Alpine divide into formerly colder areas (Studer *et al.*, 2005; IPCC, 2007).

A second goal of our survey was to try to gain insight into possible invasion/expansion routes into/within the country (and into neighbouring countries) and to identify potential sources of environmental resilience to the invasive alien. In the spirit of Primack *et al.* (2009), we were also interested in gaining insight into the ability of *R. completa* to persist in a new environment with changing climate.

Materials and methods

Collection sites and climate parameters

Samples were collected in a total of 71 sites scattered throughout Switzerland. A complete list of the localities is provided in Appendix 1, we first targeted valleys located along known paths of warm transalpine winds from the south (Föhn valleys). In particular we targeted two regions: (i) valleys in the cantons Grisons and Uri; and (ii) the flatlands of the entire canton Valais all the way from Brigg to Lake Geneva. Climatic regions, as a possible discriminating factor for incidence of *R. completa* and walnut infestation levels, were determined for individual sites on the basis of a simplified scheme following Müller (1980). We further discriminated among regions with distinct climatic conditions, such as the Jura Mountains, the midlands between Lake Geneva and Lake Constance, the north face of the Alps, the south face of the Alps beyond the Alpine divide and finally the Valais due to special conditions regarding adiabatic wind formation. Meteorological 30-year spring temperature means (March–May 1961–1990) were used as an integrating parameter reflecting growing season length and potential, respectively, and winter temperatures (December–February 1961–1990) were used as

an integrating parameter reflecting winter length and potential for survival during hibernation. Such parameters were retrieved for all walnut collection sites from the software 'Atlas of Switzerland' Version 2.0 (Project KLIMA90: Aschwanden *et al.*, 1996). Climatic parameters for each collection site are listed in Appendix 1.

For two possible south–north invasion pathways through the Gotthard region of the central Swiss Alps, yearly widths of the cold barrier unsuitable for establishment of *R. completa* around the Alpine divide were calculated from 1961 to 2010 on the basis of the homogeneous temperature series of Switzerland (Begert *et al.*, 2005) applied to the digital climate map of spring temperatures implemented with the software 'Atlas of Switzerland' Version 2.0 (Aschwanden *et al.*, 1996). Resolution of distance measurements was 1 km.

Fruit sampling and processing

Most (>95%) samples were gathered from tree canopies by direct harvest or with the aid of a 4 m telescopic scissor (expandable PVC tubing with a sharp curved scissor at end; Wallace, Enfield, CT, USA). Between 20 and 35 nuts were collected in every site (Appendix 2); but, if fruit were plentiful and heavily infested and we received explicit permission by the tree owners, up to 250 nuts were sampled. Fruit samples were placed in 10-l transparent plastic bags that were labelled (date, name of collection site) and placed in plastic crates so as to allow us to maintain the upper end of the bag open to permit for aeration.

After collection, we weighed each bag the same day and then gently transferred fruit samples inside 33×22×9-cm transparent Polystyrol containers, with a screened cover to allow for aeration (Lagerdosen PS glasklar, Semadeni AG, Ostermündingen, Switzerland). On the bottom of the container, we placed a layer of pure 0.1–0.6 mm quartz sand (minimum 93.3% SiO₂; Carlo Bernasconi AG, Bern, Switzerland) as a pupating medium. Since *R. completa* larvae wander before pupating and also creep along container walls/roof, we used three heavy duty rubber bands to guarantee that the container cover was tight, as otherwise many larvae would have escaped. In those cases where there was evidence of infestation by *R. completa* larvae, we took five such fruit from the sample for a single-fruit experiment, weighed them and placed them individually in 180-ml transparent Polystyrol cups (Joghurtbecher PS glasklar, Semadeni AG, Ostermündingen, Switzerland), into which we had also placed a layer of quartz sand in the bottom. The 180-ml cups were tightly covered with a plastic lid into which we had poked at least 100 holes with a needle to allow for aeration. Cups for the single-fruit experiment and containers with the remaining samples were then shifted to an exterior covered warehouse (i.e. ambient temperature) built with wire mesh to allow for ample aeration and protect samples from wild scavengers. Containers were randomly distributed on the warehouse subdivisions (bottom and top), avoiding stacking to allow for maximum aeration. Cups for the single-fruit experiment were placed in a plastic tray grouped by collection site.

Processing of larvae, pupae and adults

Approximately 45–60 days after fruit had been placed in the warehouse, we started to separate the pupae from the sand and debris. Fruit had totally disintegrated inside, but the

hardened skin was mostly intact and had to be cracked open to search for dead larvae or pupae. To facilitate pupal recovery, we removed all fruit and then added water to the container so as to allow pupae/dead larvae to float. Prior to this, we had carefully inspected every fruit to remove any pupae or dead larvae that remained inside skin or nut crevices. Dead larvae were surprisingly well preserved and easily recognizable as their white body contrasted with the dark liquid of the decayed pulp. At this stage, we counted all nuts in the sample as fruit had not been individually counted when originally placed in the Polystyrol containers. This information was needed to calculate the number of larvae per fruit in the overall sample.

Both larvae and pupae were transferred into a colander, rinsed with tap water and placed in 90-mm filter paper circles to let them dry. The next day at the latest, dead larvae and pupae were counted and viable pupae transferred into a 180-ml transparent Polystyrol cup with a layer of moistened quartz sand in the bottom. Some pupae were rotten or hollow and were discarded after they had been tallied so as to avoid fungal growth that could damage the healthy pupae. In the case of the single-fruit experiment, each individual pupa was weighed. After weighing, all pupae from a particular collection site that had been kept individually were placed together with those stemming from the larger sample (same location). From this pool, we separated 20 pupae (if so many were available), transferred them into 0.5-ml Eppendorf tubes and placed them at -80°C in an ultra freezer for future genetic analysis.

After the separation, rinsing and counting procedure, all pupae were transferred into a climatic chamber kept at 13°C until early January, and then the temperature was raised to 27°C to artificially break the diapause. During the test, the pupae/sand were moistened regularly. All cups containing the pupae were then transferred to a room kept at 20°C , 70% RH, 16:8 L:D cycle (long day) to monitor adult emergence. As flies emerged, they were transferred into 0.5-ml Eppendorf tubes and quickly frozen. Samples were placed together with the pupae from the same collecting site in the same ultra freezer (see previous paragraph) at -80°C for future genetic analysis.

Statistical analyses

All statistical analyses were run with the aid of XLSTAT version 2008.2.03 (Addinsoft, Aernach, Germany). The presence of *R. completa* in relation to the sampling site was analysed by multiple logistic regression to determine the influence of the climatic region within Switzerland, mean spring temperatures and mean winter temperatures with a single simultaneous analysis of incidence. The infestation level at the sites was measured as infestation per fruit and infestation per fruit mass (i.e. per kilogram of fruit). Both variables were analysed across all the sampled sites by simple factorial analyses of covariance (ANCOVA) to determine the influence of the factor climatic region and the covariates mean spring temperature and mean winter temperature. The same ANCOVA approach was applied to analyse the effect of the climate parameters on mean pupal weight measured in the overall samples (Appendix 1).

Data from the single fruit experiment were averaged for each site in order to avoid the inclusion of dependent data points in the analysis. They were first analysed to determine the possible influence of the climatic region, mean spring

temperatures and mean winter temperatures on fruit size by simple factorial ANCOVAs. The sites where no flies were found in the experiment were excluded from the analysis. Fruit weight was \log_{10} transformed in order to achieve a data structure not significantly different from the normal distribution as confirmed by Shapiro-Wilk test ($W=0.96$, $P=0.27$). Subsequently, the number of pupae per fruit was also analysed by ANCOVA to determine the possible influence of the factor climatic region, the covariates mean spring temperature, mean winter temperature and fruit weight, as well as the factor mean pupal weight in a particular fruit. Finally, mean pupal weight was analysed by ANCOVA to determine the possible influence of the factor climatic region, and the covariates mean spring temperature, mean winter temperature, fruit weight and number of pupae per fruit.

The shrinking trend of the cold barrier around the Alpine divide was tested by means of a robust non-parametric correlation based on Kendall's Tau, representing the difference between the probability that in the observed data distance is reduced over time vs. the probability that time and distance behave independently.

Results

Distribution of R. completa in Switzerland

As detailed in *fig. 1*, *R. completa* presently occurs in most lower-altitude regions of Switzerland. Of the 71 sites in which we collected walnuts, we found infested fruit in 42 sites (52.5% of total) distributed in 14 cantons. In the Ticino region, south of the Alpine divide, *R. completa* is fully established, as well as in the other smaller areas across the main mountain ridge towards Italy in the south-eastern parts of Switzerland (e.g. Bondo, Brusio). In the Valais, *R. completa* was found in the entire valley, even in the most eastern upper parts less than ten kilometres away from the Alpine divide (*fig. 1*). Also, in the canton Uri, *R. completa* occurs just about ten kilometres north of the Gotthard Massif forming the Alpine divide in this central region. North of that region, however, the species could not be found in the Lake Lucerne area. Along the upper Rhine Valley and its watershed, *R. completa* occurs only in the warm areas around Chur about 40 km north of the Alpine divide (*fig. 1*).

The incidence of *R. completa* in the sampling sites is significantly influenced by climate (multiple logistic regression, overall score $\chi^2_6=34.84$, $P<0.0001$). Among the parameters tested, however, only mean spring temperature significantly explains the incidence of *R. completa* ($\chi^2_1=8.30$, $P=0.004$), whereas climatic region ($\chi^2_4=5.40$, $P=0.25$) and mean winter temperature ($\chi^2_1=0.013$, $P=0.91$) had no effect. The regions where the fly is not present but the host (*J. regia*) is are located in the central part of the country in areas where the 30-year mean spring temperature fluctuates between 5°C and about 7°C , or falls below these mean values (cantons Glarus, Luzern, Nidwalden, Obwalden, Schwyz, and parts of Bern, Grisons and Jura) (Appendix 1).

Infestation patterns

Fruit infestation levels varied greatly between the sites sampled and spanned from 0 to 259.32 larvae per kilogram of fruit (Appendix 1). Although the maximum infestation levels were detected in the outskirts of the city of Bern within the central midlands of the northern pre-alpine region, heavily

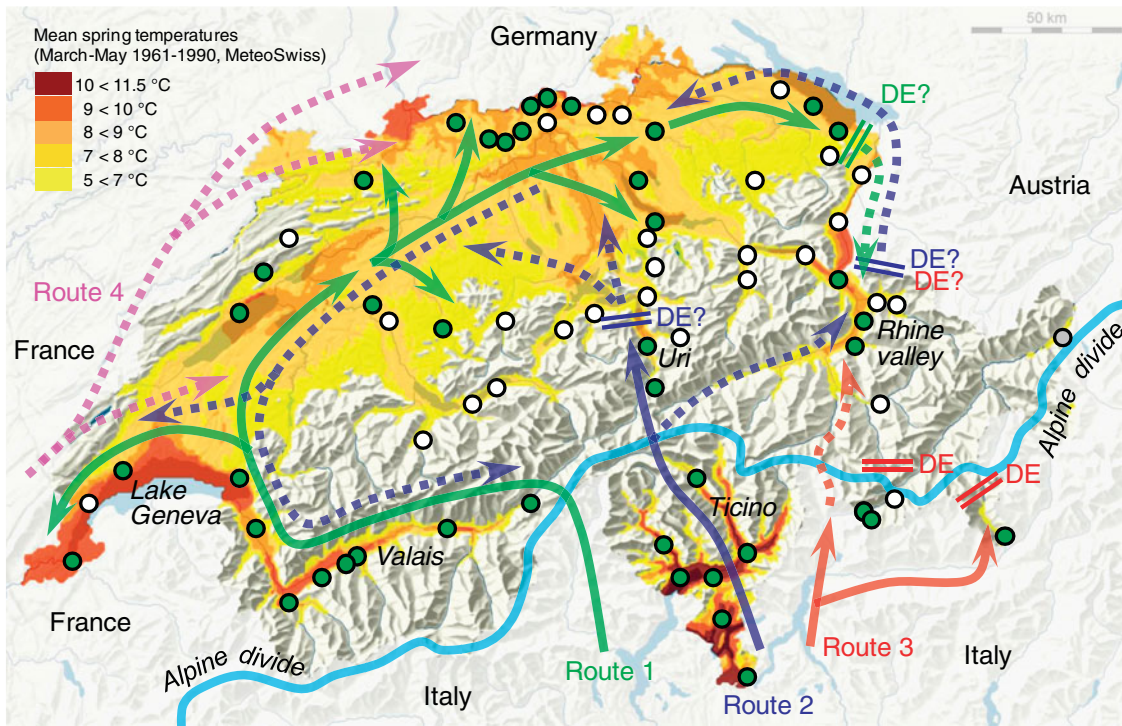


Fig. 1. Current distribution of *R. completa* in Switzerland with possible invasion and expansion routes. Green circles represent sites where infested fruits were collected, and white circles represent sites where fruits were collected but no infestation was determined. The grey circle represents the locality of Martina (Grisons) where no walnut trees were found despite suitable altitude and climatic conditions for tree survival. Alpine divide drawn as main physical and climatic barrier (light blue line). Four possible invasion/expansion routes are depicted: green arrows (most likely), blue (likely), pink (possible) and red (unlikely). DE, dead end (i.e. at present not yet passed physical/climatic barrier). Graphics by J. Samietz.

infested fruit was also collected in various other sites distributed all over Switzerland, such as Brusio (Grisons), Courrendin (Jura), Chur (Grisons) and Etzgen (Aargau) (details in fig. 2 and Appendix 1). The number of larvae per fruit varied between 0.08 and 9.88, averaging 1.55 for all infested samples (Appendix 1).

Infestation level per fruit was significantly influenced by mean spring temperature (ANCOVA, $F_{1,68}=4.97$, $P=0.029$). Climatic region and mean winter temperature, however, had no significant effect (ANCOVA, factor: $F_{4,68}=1.32$, $P=0.274$; covariate: $F_{1,68}=2.70$, $P=0.106$). Thus, the discriminating influence of spring temperature exhibits the same pattern in all climatic regions (no interaction).

Infestation rate per fruit mass was likewise significantly influenced by mean spring temperature (ANCOVA, $F_{1,68}=5.49$, $P=0.022$). Climatic region and mean winter temperature had no significant effect (ANCOVA, factor: $F_{4,68}=2.41$, $P=0.059$; covariate: $F_{1,68}=3.65$; $P=0.061$). As was the case for infestation level per fruit, the discriminating influence of spring temperature exhibits the same pattern in all climatic regions (no interaction).

The effects of seasonal temperatures on infestation level per fruit and infestation rate per fruit mass were confirmed when analysed separately. Whereas infestation by *R. completa* was not related to winter temperatures (per fruit: Spearman's $r=0.307$, $P=0.111$; per fruit mass: Spearman's $r=0.315$, $P=0.092$), infestation rates were significantly correlated with spring temperatures (fig. 3a, per fruit: Spearman's $r=0.575$,

$P<0.0001$; fig. 3b, per fruit mass: Spearman's $r=0.577$, $P<0.0001$).

Pupae weighed, on average, 7.66 ± 0.21 mg (Appendix 1) and weight was not influenced by any of the climatic parameters analysed (ANCOVA, factor climatic region: $F_{4,39}=0.198$, $P=0.938$; covariates: spring temperature: $F_{1,39}=0.007$, $P=0.934$; winter temperature: $F_{1,39}=1.34$, $P=0.255$).

Single-fruit experiment

In the case of the subsample of walnuts that were kept individually after harvest, individual fruit weight ranged from 19.54 g, for a nut sampled in Aigle (Valud), to 119.2 g, for a nut sampled in Le Guercet (Valais); but this parameter was not influenced by the climatic parameters analysed (\log_{10} -transformed; ANCOVA, factor climatic region: $F_{4,159}=0.694$, $P=0.603$; covariates: spring temperature: $F_{1,159}=1.44$, $P=0.241$; winter temperature: $F_{1,159}=0.164$, $P=0.689$). For mean fruit mass in the samples, refer to Appendix 2.

Infestation level measured as the number of pupae per fruit was significantly influenced by individual fruit weight (\log_{10} -transformed; ANCOVA, $F_{1,31}=8.67$, $P=0.008$) but not by mean pupal weight nor any of the climatic parameters analysed (ANCOVA, factor climatic region: $F_{4,31}=0.379$, $P=0.821$; covariates: spring temperature: $F_{1,31}=2.28$, $P=0.146$; winter temperature: $F_{1,31}=0.059$, $P=0.810$; pupal weight: $F_{1,31}=0.016$, $P=0.901$). The discriminating influence of fruit weight on infestation per fruit exhibits the same pattern in all

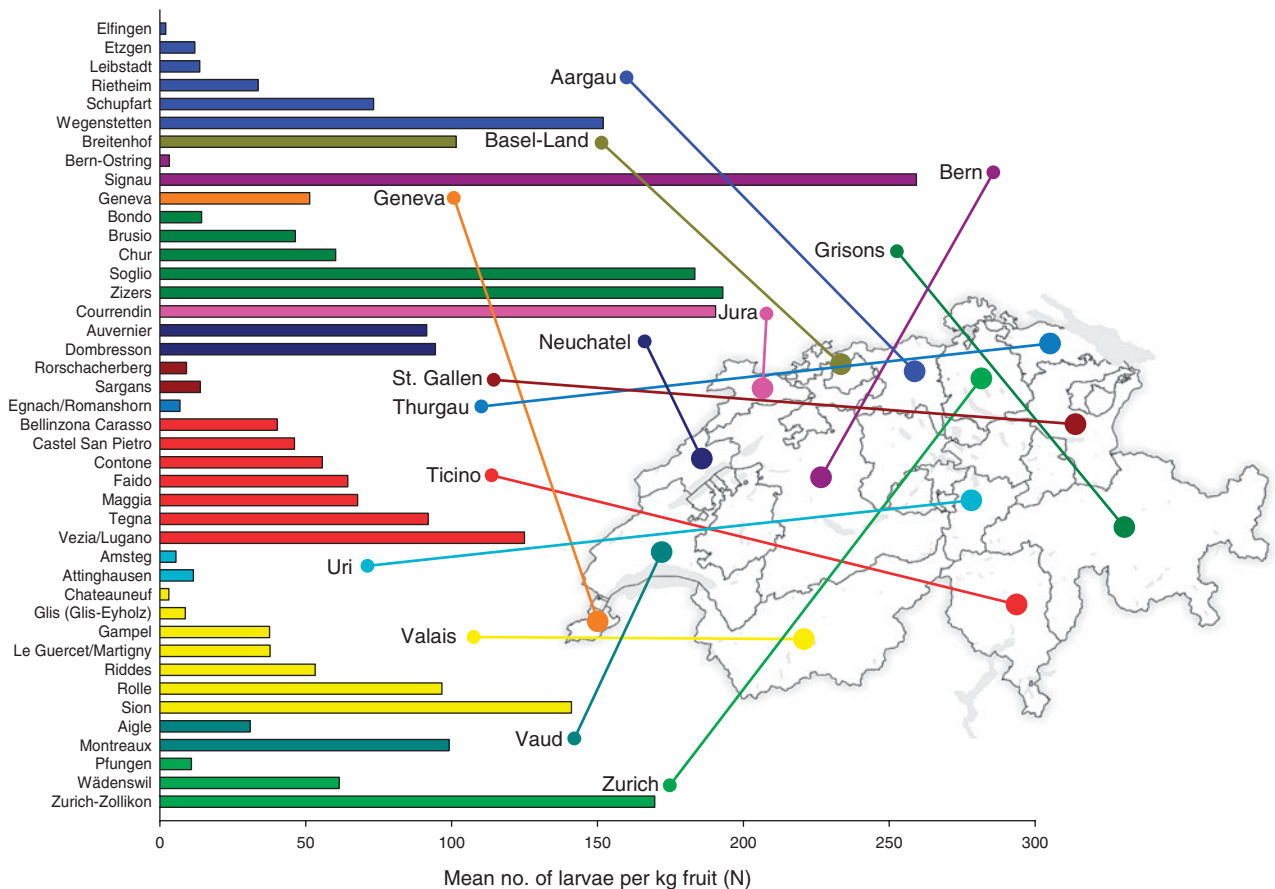


Fig. 2. Degree of infestation (larvae per kg of fruit) in infested localities across Swiss cantons, illustrating the fact that sites with the highest levels of infestation by *R. completa* show no discernible geographical pattern. Graphics by J. Samietz.

climatic regions (no interaction). The corresponding relationship between infestation per fruit and \log_{10} -transformed fruit weight is plotted in fig. 4 (for regression statistics, see graph).

Weight of single pupae in individually kept fruit varied from 4.30 mg in Contone (Ticino) to 10.32 ± 0.26 mg in Bondo (Grisons), both located south of the Alpine divide (Appendix 2). Overall (considering all fruit kept individually independent of site of collection), pupae weighed a mean 7.66 ± 0.21 mg. Importantly, pupal weight was neither influenced by individual fruit weight and pupal number per fruit nor by any of the climatic parameters analysed (ANCOVA, factor climatic region: $F_{4,31} = 0.624$, $P = 0.657$; covariates: spring temperature: $F_{1,31} = 0.024$, $P = 0.879$; winter temperature: $F_{1,31} = 1.00$, $P = 0.329$; infestation pupae per fruit: $F_{1,31} = 0.0038$, $P = 0.956$; \log_{10} -transformed fruit weight: $F_{1,31} = 3.52$, $P = 0.559$).

Based on the above, it becomes clear that there is no trade-off between fruit size, fruit infestation level and pupal weight. To illustrate the link between the variables, pupal weight was plotted as a function of \log_{10} -transformed fruit size and degree of infestation in fig. 5. The nearly horizontal three-dimensional least-square mesh visualizes and confirms the ANCOVA results. That is, the smallest pupae did not stem from the most infested or smallest fruit (fig. 5).

Parasitism

A total of 6476 pupae were processed in the entire study. Of these pupae, 3675 were followed until break of diapause and adult fly emergence. In the case of the rest, we only checked for parasitoid emergence. Not a single parasitoid emerged in any case.

Alpine divide as a climatic barrier

From 1961 to 1990, in the Gotthard region of the central Swiss Alps, the width of the cold barrier around the Alpine divide, based on $\leq 7^\circ\text{C}$ spring temperatures unsuitable for establishment of *R. completa*, was on average 43.0 ± 26.0 km between the Ticino Valley (south) and the Reuss Valley (north) and 38.4 ± 16.0 km between the Brenno Valley (south) and the Rhine Valley (north). In the past ten years (2001–2010), the width for the possible south-north invasion pathways shrunk to only 21.8 ± 5.6 km between the Ticino and Reuss Valleys and 19.6 ± 5.7 km between the Brenno and Rhine Valleys (fig. 6). During 2007, a record year with respect of spring temperatures, the shortest width was reached with only 11 km between sites with favourable temperatures across the Brenno and Rhine Valleys (fig. 6). The observed trend of shrinking climatic barrier is highly significant for both analysed

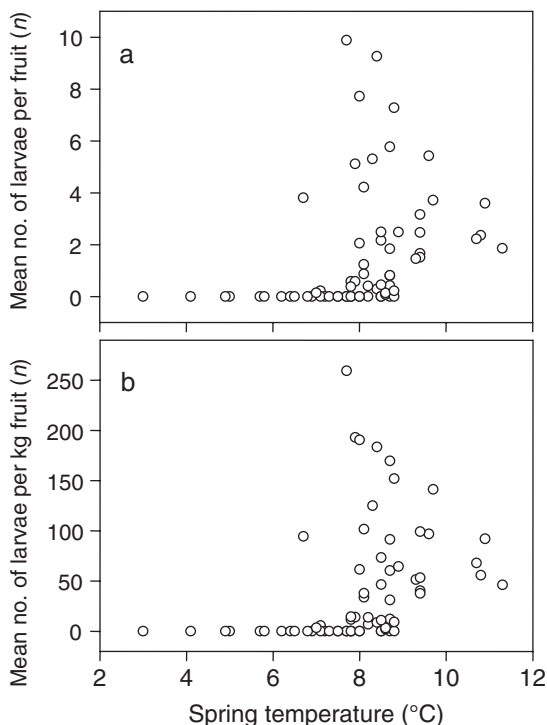


Fig. 3. Level of infestation of *R. completa* (a) per walnut fruit sampled and (b) per kilogram fruit as linear functions of meteorological 30-year averages of spring temperatures (March–May) in Switzerland on 69 locations. Refer to the text for statistics.

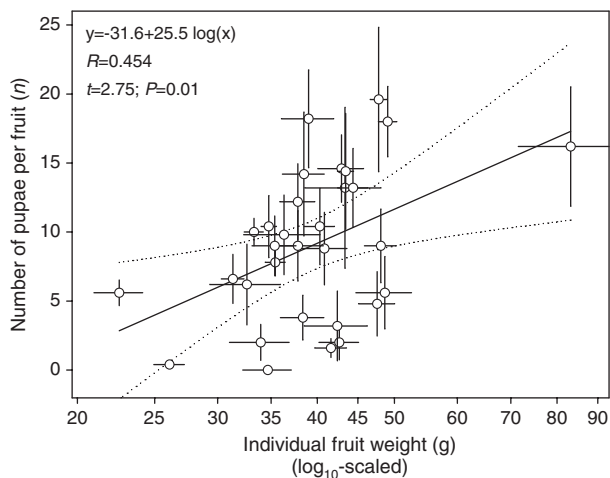


Fig. 4. Level of infestation of *R. completa* per walnut fruit as linear function of individual fruit weight in a single-fruit experiment ($n=160$) with samples from 32 locations in Switzerland. Dashed lines: 95% confidence of the regression slope. Dotted lines: 95% confidence of the prediction range.

pathways across the Gotthard region (Ticino-Reuss Valley: Kendall's Tau = -0.469 , $P < 0.0001$; Brenno-Rhine Valley: Kendall's Tau = -0.462 , $P < 0.0001$).

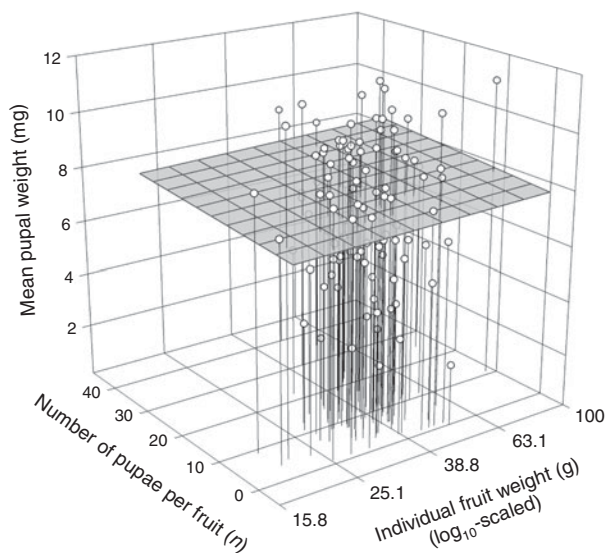


Fig. 5. Mean pupal weight of *R. completa* as multiple linear function of individual fruit weight and infestation level per walnut fruit in a single-fruit experiment ($n=160$) with samples from 32 locations in Switzerland. Mesh shows three-dimensional least-square relationship which reveals no effect of the two parameters (for statistics see text).

Discussion

Several findings from this study merit discussion. (i) *R. completa* is now firmly established in most of Switzerland. The only areas still free of the pest are those where mean spring temperatures fall below 7°C or in all mountainous areas where walnut trees cannot grow. We propose factors possibly explaining its current distribution in this country and also hypothesize on possible invasion/expansion routes within Switzerland and to neighbouring countries. (ii) The sites exhibiting the highest rates of infestation are distributed all over Switzerland, with no discernible geographical pattern. (iii) We did not find a significant correlation between the degree of infestation (i.e. pupae per fruit) and pupal weight and between fruit weight and pupal weight (i.e. the smallest pupae did not stem from the most infested or smallest fruit). We discuss all the above to raise awareness on the risk of invasion of other pestiferous fruit fly species that pose potential threats, such as the apple maggot, *R. pomonella*, and other species in the same genus or, in the longer term, tropical species, such as the Medfly or the Oriental fruit fly (Meixner *et al.*, 2002; Clarke *et al.*, 2005).

Possible invasion and expansion routes

A recent literature review revealed that most biological invasions in Canada follow a defined pattern of introduction and spread, with some species being directly introduced but many others expanding their range from original entry points in the US (Langor *et al.*, 2009). Identifying invasion routes becomes, therefore, a highly relevant endeavour. Incurion of invasive species from the Mediterranean across the Alps is most likely limited by the climatic limits of the species and by the orographic structure of the alpine region – especially by the Alpine divide and the main valleys. Mountain ranges

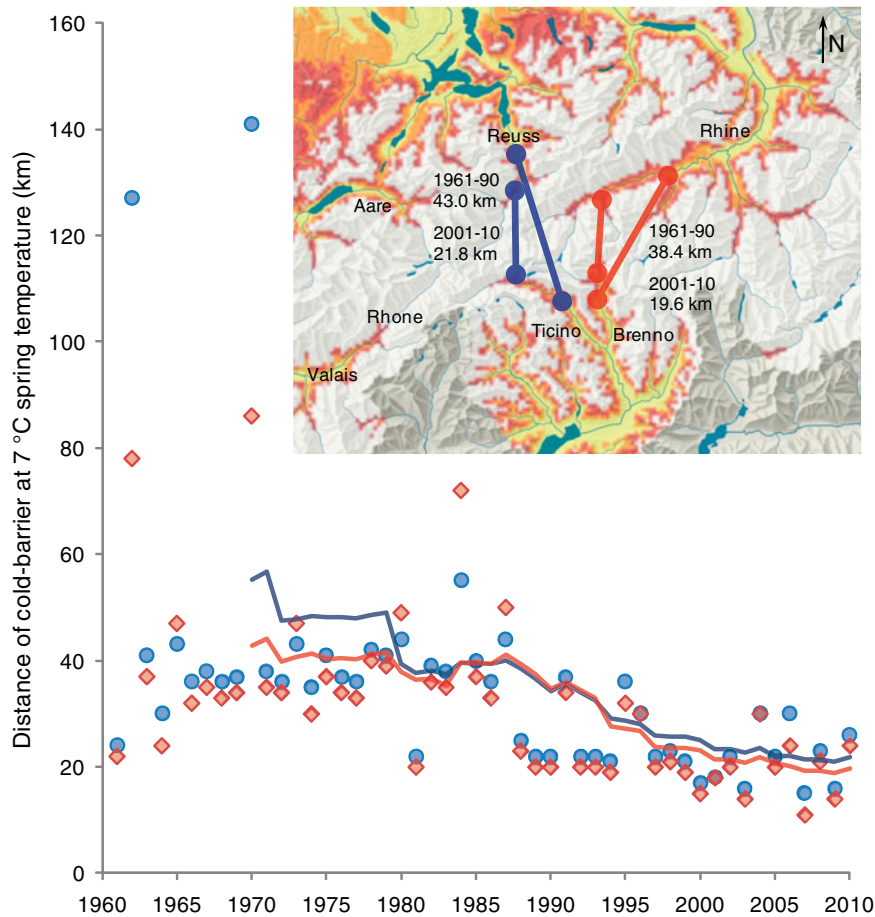


Fig. 6. Distance of cold barrier around the Alpine divide based on 7°C spring temperature from 1961 to 2010 for two possible south–north invasion pathways through the Gotthard region (Ticino – Reuss; Brenno – Rhine). Lines show ten-years running mean. Map insert depicts areas with mean spring temperatures for 1961–90 (yellow), 1989–2010 (orange), 2001–2010 (Red), and the record year 2007 (dark red). Graphics by J. Samietz (●, Ticino – Reuss valley; ◆, Brenno – Rhine valley).

create a natural climatic and physical barrier that somehow had to be crossed by *R. completa* since it was first reported in Europe in northern Italy (Duso, 1991; Ciampolini & Trematerra, 1992). In fig. 1, we propose four possible routes of entry of *R. completa* into Switzerland and for its expansion within the country. At this stage of the analysis, we ignore if another pathway for entry into the country could have potentially been the incidental or accidental importation of infested fruit by car. Such founder events will be explored by us in subsequent studies on the population genetics of *R. completa* since they have been found to leave genetic signatures (Berlocher, 1984; Chen *et al.*, 2006).

Given the widespread distribution in the Valais, even in the upper valley less than ten kilometres away from the Alpine divide, a very plausible transalpine crossing point is represented by Route 1 (fig. 1; green arrows) through which adult flies would have crossed the Simplon area into the upper Valais from northern Italy. This would also be supported by Föhn winds occurring in that region that can form quite strong and warm adiabatic currents. Additionally, the Valais is a narrow valley in itself, where strong adiabatic winds that change direction during the day are generated, possibly aiding adults to quickly expand from the upper Valais towards Lake

Geneva. In both cases, flies could also have been introduced via infested fruit by nursery owners driving into the country by car.

Another very likely incursion route into the southern Ticino and plausible alpine crossing is represented by Route 2 (fig. 1; blue arrows). Adult flies likely moved into Switzerland from northern Italy along the prealpine lakes in that area (Lugano and Maggiore). Once established in the Ticino, a region where walnuts are plentiful and widespread, adult flies may have crossed the Alpine divide through the narrow Gotthard Pass into the Canton of Uri, where they could have established founder populations. Again, this may have been supported by the strong Föhn winds occurring there in the case of southern currents. From Uri, a canton where walnuts are also common in farms, backyard gardens and parks, flies could have expanded into the rest of the country via the cantons of Schwyz, Zürich, Grisons and St Gallen. Initial expansion from the Canton of Uri into northern Switzerland could have occurred via the Lake Lucerne and the Reuss Valley. We note, however, that we did not find *R. completa* directly north of Uri and in sites around Lake Lucerne. So, it is also possible that founder populations in Uri stemming from the Ticino may have disappeared after a harsh winter; and,

if such would have been the case, Route 2 may well represent a dead end.

A third potential alpine crossing, albeit a non-parsimonious one, is represented by Route 3 (fig. 1; red arrows), where flies would have entered the Rhine Valley and its watershed from the Lake Como region in Italy via the Splügen Pass, aided by Föhn winds and then moved into St Gallen, Aargau, Schaffhausen, Basel and the Jura, Neuchâtel and Valais (opposite direction as Route 1). However, the locations in which we discovered *R. completa* populations (Chur, Zizers) are quite distant from the Alpine divide (40 km), and we therefore consider this hypothesis as weak. It is also possible that the flies found in western Grisons arrived via invasion routes one or two (or both).

Finally, Route 4 (fig. 1; pink arrows) would suppose that *R. completa* crossed the Alps in southern France or at least in the southernmost and, thereby, warmer parts of the Alpine divide and then would have invaded Switzerland through the Rhone valley. Although the presence of *R. completa* in France has only been recently formally acknowledged (EPPO, 2008; Bouvet, 2009), it is likely that invasion into this country occurred a long time ago and that the species is now firmly established as being the case in Switzerland. Recent invasion of the species into Germany (EPPO, 2004) likely happened by crossing the Alpine divide, either via France or more likely by one of the hypothesized routes through Switzerland. At least, the first unofficial reports of the presence of *R. completa* in Germany stem from localities in southern Baden – the tri-border region of France, Germany and Switzerland (Kirsten Köppler, personal communication). Of course, it is also possible that infested walnuts could have been carried into the country by car. In our planned genetic analysis of European populations, the genetic structure of *R. completa* populations will be analysed along the lines of Meixner *et al.* (2002) and Michel *et al.* (2007), including reference sampling in the areas of origin, to further elucidate the dynamics of the Swiss invasion.

Considering all the above, global warming over the past 50 years, and especially after 1990, appears to have facilitated the crossing of *R. completa* over the highest regions of the Swiss Alps. As analysed here, from 1985 on, there is a progressive reduction of the dividing effect around the Alpine divide (fig. 6). The ten-year running means of both pathways analysed here progressively fell from 1987 on, about the time when *R. completa* was introduced into Europe. When considering recent climate change scenarios, during the next two to three decades this climatic barrier will further shrink to mean width values of about 15 km (Samietz, unpublished data; IPCC, 2007). But even considering current crossing distances – on average only about 20 km and as little as 11 km in record years (e.g. 2007) – Alpine crossing aided by Föhn winds or even by active flight has become much more likely than it was only some decades ago. This should be taken into account when monitoring species establishment in the Mediterranean Basin and, thereafter, in the Alpine valleys.

The lack of a clear geographical pattern with respect to levels of infestation could be indicative of the fact that local microclimate influences larval development. However, results from the single-fruit experiment clearly indicate that fruit weight is positively correlated to infestation level by *R. completa* (fig. 4). That is, large fruit provide more resources for feeding larvae and, therefore, produce more pupae and eventually adults. Nevertheless, pupal weight, a key fitness parameter, shows no trade-off with fruit size and degree of

infestation (fig. 5). That is, the smallest pupae did not necessarily stem from fruit that harboured the largest number of larvae. Therefore, we believe that the observed infestation patterns can be better explained by varying levels of secondary metabolites (allelochemicals) in fruit, in turn determined by cultivar, age of tree or by growing conditions, rather than microclimate. In support of the above, differences in walnut cultivar susceptibility to *R. completa* have been reported in California (Boyce, 1934; Hass, 1937; Opp & Zermeño, 2001; Coates, 2005). We believe that until more accurate information on the effect of cultivar (plant chemistry) can be gathered, presence absence data, rather than infestation levels, is better suited to make inferences on potential invasion routes.

Monitoring and management of invasion

Considering the fact that two Nearctic pest species of *Rhagoletis* have invaded Switzerland and neighbouring countries in the past 20–30 years, preventive detection trapping for *Rhagoletis pomonella* a key pest of apples, *R. indifferens* a key pest of cherries, *R. fausta* a potential pest of cherries, and other *Rhagoletis* species in the *suaavis* group as potential pests of walnuts should be established along probable invasion routes. An advantage, in this respect, is that all of these species are visually attracted to yellow panels, and immature adults of all species respond to ammonium carbonate (Aluja & Rull, 2009). Therefore, a single trapping device could be employed to monitor all of these species. We also believe that if global warming trends persist, potentially allowing tropical species to survive in formerly impenetrable areas because of the presence of permanent snow or ice (and concomitant cold air), preventive trapping routes, placed along strategic valleys such as the Valais to detect the potential presence of species such as *B. dorsalis*, *C. capitata* or *A. fraterculus*, may be warranted.

In the USA, yellow traps baited with ammonium acetate are recommended to monitor *R. completa* populations (Riedl & Hoying, 1981; Riedl *et al.*, 1989). Once the first adult flies emerge, insecticides such as spinosad and malathion have been applied in California to entire tree canopies (Riedl *et al.*, 1989; Van Steenwyk *et al.*, 2003). An organic alternative to insecticides applied in California is represented by clay-based kaolin products (e.g. Surround[®]) (Coates & Van Steenwyk, 2002). Among the European countries recently invaded by *R. completa*, France, a country with large commercial walnut-producing areas, has approved the restricted use (limit of 120 days) of thiacloprid and phosmet for conventional agriculture and spinosad for organic nut production in 2009 (guidelines of EU Directive 2000/29, Annex A) (Bouvet, 2009).

The potential danger *R. completa* represents to peach growers in Central Europe is a topic that merits attention. This fly has been shown to attack peaches in California, where, fortunately, its diapause schedule causes it to emerge at the end of the peach harvest season, allowing for the implementation of a 'pest-free harvest period' (Yokoyama & Miller, 1993, 1994). Nevertheless, it has to be kept in mind that plasticity in diapause length facilitated expansion of *R. completa* from its native range in the midwestern US (5°C) to California, where warmer winter temperatures prevail (10°C) (Chen *et al.*, 2006). *R. completa* may possess the genetic variability necessary to evolve diapause lengths to match the fruiting phenology of potential European host plants. We, therefore, suggest the establishment of a preventive monitoring network to help reduce the potential danger posed by *R. completa* to peach growers in Hungary and Romania,

as this species has already been detected in Austria and Slovenia (Seljak, 1999; Lethmayer, 2008).

Insights into the biology and ecology of Rhagoletis completa

Our study yielded many insights into the biology and ecology of this invasive pest and complements nicely the work of pioneers such as Cresson (1929) and Boyce (1934) (for a more recent review see Prokopy & Papaj, 2000). For example, quantitative data on rates of infestation were retrieved (e.g. larvae per fruit), which in turn shed light into the oviposition behaviour of females, which, as far as is known, lay one egg per oviposition bout but commonly reuse previously used oviposition punctures. Our findings coincide with those of Papaj (1994) and Nufio *et al.* (2000) who, working with other walnut infesting species (*R. boycei* and *R. juglandis*), also report the presence of multiple larvae per fruit, the result of multiple ovipositions by the same female or conspecifics. Our discoveries on the close temperature relationship between *R. completa* and sites where it is present expand our knowledge on the ecology of this tephritid fly, as previously pointed out by Chen *et al.* (2006) in California for introduced populations of *R. completa* and by Filchak *et al.* (2000) for host races of *R. pomonella*. This is important, as gene–environment interactions related to the effect of local environment and host phenology can produce genetically differentiated populations adjusted to different hosts (Berlocher, 1984).

As is common with invasive species (Torchin *et al.*, 2003), we found that parasitism is currently nil in Switzerland. That is, the pest escaped from an important mortality factor, at least for a while. Previously, Feder (1995), working with the apple maggot, *R. pomonella*, documented the fact that, by shifting hosts (from the native hawthorn (*Crataegus mollis* (Torr. & A. Gray) Scheele) to the introduced apple (*Malus domestica* Borkh.)), the degree of parasitism fell from a mean of 46% to a mean of 13%. Similarly, *R. completa* is attacked by *Aganaspis alujai* in its native range, while no parasitoids have ever been recorded in association with this species in California (Boyce, 1934; Legner & Goeden, 1987; Ovruski *et al.*, 2007).

In conclusion, we were not only able to firmly establish the current distribution of *R. completa* in Switzerland but gained significant insights into its biology and ecology, which will become handy when management schemes in the invaded European countries have to be designed. Additionally, our findings here highlight the rapid pace with which *Rhagoletis* pest species of Nearctic origin can establish and expand within Europe with perhaps global warming allowing crossing of the Alps much more rapidly than it was the case some decades ago. *Rhagoletis cingulata* is supposed to have a similar invasive history as *R. completa* and currently is becoming a nuisance in sour-cherry growing regions of Germany and neighbouring countries (K. Köppler, personal communication). Considering current global warming trends and particularly the ever increasing volume of international trade, additional invasions represent a realistic potential threat that should be taken seriously (Work *et al.*, 2005). Therefore, identifying potential entry routes, climate parameters promoting or hindering expansion, potential host species, better understanding of environmental resilience to invasive species and prospecting remedial measures should become a high priority to minimize potential damage to fruit production in European countries by other potentially invasive fruit fly species.

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Appendix 1. Survey results for *Rhagoletis completa* in Switzerland organized according to climatic region and decreasing mean spring temperatures within region. Spring temperatures: March–May 1960–1990; winter temperatures: December–February 1961–1990.

Locality	Canton	Climate Region	Temperature (°C)		Sampling date	Quantity/ No. Fruits		Infestation	Mean No. larvae per		Mean pupal weight (mg)
			Spring	Winter		Mass (kg)	No.		Fruit	kg Fruit	
Tegna	Ticino	Alps south	11.3	4.3	8 Sep 08	3.898	97	Yes	1.86	46.18	6.77
Bellinzona Carasso	Ticino	Alps south	10.9	2.7	8 Sep 08	2.110	54	Yes	3.59	91.94	8.40
Contone	Ticino	Alps south	10.8	2	8 Sep 08	3.805	90	Yes	2.36	55.72	7.16
Vezia/Lugano	Ticino	Alps south	10.7	2.8	8 Sep 08	3.199	98	Yes	2.21	67.83	7.48
Castel San Pietro	Ticino	Alps south	9.4	0.6	8 Sep 08	1.937	47	Yes	1.66	40.27	9.80
Maggia	Ticino	Alps south	8.9	3.5	8 Sep 08	3.772	98	Yes	2.48	64.42	6.32
Bondo	Grisons	Alps south	8.7	-1.7	30 Aug 08	2.588	85	Yes	1.84	60.28	7.47
Faido	Ticino	Alps south	8.3	0.6	8 Sep 08	3.225	76	Yes	5.30	124.96	10.44
Brusio	Grisons	Alps south	7.9	0.5	30 Aug 08	2.197	83	Yes	5.11	192.99	8.85
Soglio	Grisons	Alps south	7.8	-4.5	30 Aug 08	1.885	72	Yes	0.38	14.32	9.75
Vicosoprano	Grisons	Alps south	3	-2.9	20 Sep 08	0.352	10	No	0.00	0.00	
Sargans	St. Gallen	Alps north	8.8	0.5	24 Sep 08	0.548	23	Yes	0.22	9.12	
Zizers	Grisons	Alps north	8.5	0.3	24 Sep 08	1.873	35	Yes	2.49	46.45	9.63
Chur	Grisons	Alps north	8.4	0.1	24 Sep 08	2.121	42	Yes	9.26	183.40	8.78
Grabs	St. Gallen	Alps north	8	1.4	24 Sep 08	0.972	25	No	0.00	0.00	
Walenstadt	St. Gallen	Alps north	8	1	24 Sep 08	0.794	33	No	0.00	0.00	
Attinghausen	Uri	Alps north	7.8	1	23 Sep 08	1.218	24	Yes	0.58	11.49	11.04
Ingenbohl, Brunnen	Schwyz	Alps north	7.8	0.9	23 Sep 08	0.996	20	No	0.00	0.00	
Näfels	Glarus	Alps north	7.8	0	24 Sep 08	0.853	26	No	0.00	0.00	
Schiers (dir. Landquart)	Grisons	Alps north	7.8	-0.5	24 Sep 08	1.274	36	No	0.00	0.00	
Muri	Bern	Alps north	7.7	0	23 Sep 08	1.463	38	No	0.00	0.00	
Interlaken	Bern	Alps north	7.7	-0.2	8 Oct 08	0.833	50	No	0.00	0.00	
Stans	Nidwalden	Alps north	7.5	2.6	23 Sep 08	1.089	29	No	0.00	0.00	
Sarnen, Föribach	Obwalden	Alps north	7.3	2.3	7 Oct 08	1.022	34	No	0.00	0.00	
Glarus	Glarus	Alps north	7.2	-0.4	24 Sep 08	0.920	24	No	0.00	0.00	
Lüchingen	St. Gallen	Alps north	7.1	1.4	24 Sep 08	1.246	28	No	0.00	0.00	
Amsteg	Uri	Alps north	7.1	1.2	23 Sep 08	0.908	23	Yes	0.22	5.51	5.78
Signau	Bern	Alps north	7	-0.5	23 Sep 08	1.223	29	Yes	0.14	3.27	8.10
Kien	Bern	Alps north	6.9	-0.2	8 Oct 08	- ¹	-	No	-	-	
Niederried/Interlaken	Bern	Alps north	6.8	-1.1	8 Oct 08	1.235	63	No	0.00	0.00	
Schüpfheim	Luzern	Alps north	6.4	0.8	23 Sep 08	0.808	21	No	0.00	0.00	
Sattel	Schwyz	Alps north	5.8	-1.3	23 Sep 08	0.864	21	No	0.00	0.00	
Maria/Schiers	Grisons	Alps north	5.7	-2.3	24 Sep 08	0.384	16	No	0.00	0.00	
Martina	Grisons	Alps north	5.2	-3.5	30 Sep 08	- ²	-	No	-	-	
Surava	Grisons	Alps north	4.9	-0.4	30 Aug 08	0.032	1	No	0.00	0.00	

Appendix 1. (Cont.)

Locality	Canton	Climate Region	Temperature (°C)		Sampling date	Quantity/ No. Fruits		Infestation	Mean No. larvae per		Mean pupal weight (mg)
			Spring	Winter		Mass (kg)	No.		Fruit	kg Fruit	
Spiringen/Locherbach	Uri	Alps north	4.1	-3.4	7 Oct 08	0.877	27	No	0.00	0.00	
Riddes	Valais	Valais	9.7	1.9	9 Sep 08	1.552	59	Yes	3.71	141.11	7.63
Le Guercet/Martigny	Valais	Valais	9.6	1.7	9 Sep 08	3.868	69	Yes	5.42	96.69	9.48
Chateauneuf	Valais	Valais	9.4	0.6	9 Sep 08	2.288	57	Yes	1.51	37.59	8.42
Sion	Valais	Valais	9.4	0.2	9 Sep 08	3.888	84	Yes	2.46	53.24	6.56
Aigle	Vaud	Valais	8.7	1	9 Sep 08	0.646	25	Yes	0.80	30.96	9.03
Rolle	Valais	Valais	8.6	1.8	30 Sep 08	3.886	89	Yes	0.13	3.09	
Gampel	Valais	Valais	8.4	0.1	8 Oct 08	1.720	53	Yes	0.28	8.72	7.16
Glis (dir. Eyholz)	Valais	Valais	8.1	-0.3	8 Oct 08	0.874	38	Yes	0.87	37.76	7.32
Montreaux	Vaud	Midlands	9.4	2.4	9 Sep 08	1.402	44	Yes	3.16	99.14	10.53
Geneva	Geneva	Midlands	9.3	2	9 Sep 08	1.753	62	Yes	1.45	51.34	8.19
Nyon	Vaud	Midlands	8.8	1.5	30 Sep 08	0.744	33	No	0.00	0.00	
Etzgen	Aargau	Midlands	8.8	1.2	17 Sep 08	1.053	22	Yes	7.27	151.95	7.62
Zürich-Zollikon	Zurich	Midlands	8.7	0.9	2 Oct 08	8.667	255	Yes	5.76	169.61	9.20
Kaiserstuhl	Aargau	Midlands	8.7	0.4	17 Sep 08	1.481	33	No	0.00	0.00	
Rietheim	Aargau	Midlands	8.7	0.4	17 Sep 08	1.083	31	Yes	0.42	12.00	7.44
Leibstadt	Aargau	Midlands	8.6	0.7	17 Sep 08	1.457	40	Yes	0.08	2.06	7.00
Schupfart	Aargau	Midlands	8.5	1.2	17 Sep 08	1.092	37	Yes	2.16	73.26	10.53
Hottwil	Aargau	Midlands	8.5	0.7	17 Sep 08	1.269	41	No	0.00	0.00	
Wädenswil	Zurich	Midlands	8.5	0.7	22 Sep 08	1.937	47	Yes	0.45	10.84	7.53
Glattfelden	Zurich	Midlands	8.5	0.3	17 Sep 08	0.644	30	No	0.00	0.00	
Wegenstetten	Aargau	Midlands	8.2	1	17 Sep 08	0.875	30	Yes	0.40	13.71	7.27
Egnach/Romanshorn	Thurgau	Midlands	8.2	0.6	24 Sep 08	1.160	26	Yes	0.00	6.90	7.03
Breitenhof	Basel-Land	Midlands	8.1	0.6	17 Sep 08	1.950	47	Yes	4.21	101.54	7.32
Elfingen	Aargau	Midlands	8.1	0.3	17 Sep 08	0.951	26	Yes	1.23	33.65	6.97
Pfungen	Zurich	Midlands	8	0.2	17 Sep 08	1.237	37	Yes	2.05	61.44	8.95
Rorschacherberg	St. Gallen	Midlands	7.9	0.7	24 Sep 08	1.083	26	Yes	0.58	13.85	8.73
Bern-Ostring	Bern	Midlands	7.7	0	23 Sep 08	1.905	50	Yes	9.88	259.32	8.80
Langrickenbach	Thurgau	Midlands	7.5	-0.1	24 Sep 08	2.098	52	No	0.00	0.00	
Hirzel	Zurich	Midlands	6.9	-0.5	9 Oct 08	0.968	48	No	0.00	0.00	
St. Gallen	St. Gallen	Midlands	6.5	-0.7	24 Sep 08	1.419	35	No	0.00	0.00	
Lichtensteig	St. Gallen	Midlands	6.2	-1.1	24 Sep 08	1.201	39	No	0.00	0.00	
Auvernier	Neuchatel	Jura	8.7	1.6	10 Sep 08	0.667	75	Yes	0.81	91.45	7.88
Courrendin	Jura	Jura	8	0.4	10 Sep 08	1.134	28	Yes	7.71	190.48	7.76
Dombresson	Neuchatel	Jura	6.7	0.1	10 Sep 08	1.895	47	Yes	3.81	94.46	9.00
St. Imer	Bern	Jura	5	0.4	10 Sep 08	0.477	10	No	0.00	0.00	

¹ Sampling impossible but no obvious infestation.² no nut trees albeit sufficient growing conditions.

Appendix 2. Infestation patterns of *Rhagoletis completa* in the single-fruit experiment (five fruits per locality) listed in the same order as in table 1.

Locality	Climate	Temperature (°C)		Fruit weight (g)		Pupae per fruit (<i>n</i>)		Pupal weight (mg)	
		Spring	Winter	Mean	SE	Mean	SE	Mean	SE
Tegna	Alps south	11.3	4.3	48.64	3.91	5.60	2.62	4.58	1.22
Bellinzona Carasso	Alps south	10.9	2.7	40.30	1.75	10.40	2.77	6.63	1.76
Contone	Alps south	10.8	2	42.64	2.45	2.00	1.26	4.30	
Vezia/Lugano	Alps south	10.7	2.8	38.37	2.42	3.80	1.62	8.15	0.78
Castel San Pietro	Alps south	9.4	0.6	43.34	4.76	13.20	5.83	7.22	0.87
Maggia	Alps south	8.9	3.5	40.83	2.76	8.80	2.63	5.21	1.11
Bondo	Alps south	8.7	-1.7	31.34	1.03	6.60	1.78	10.32	0.26
Faido	Alps south	8.3	0.6	39.01	2.93	18.20	3.54	7.10	
Brusio	Alps south	7.9	0.5	33.31	0.91	10.00	1.00	7.89	0.97
Soglio	Alps south	7.8	-4.5	35.56	0.95	0.00			
Zizers	Alps north	8.5	0.3	47.79	1.16	19.60	5.23	9.33	0.68
Chur	Alps north	8.4	0.1	48.08	2.23	9.00	2.66	8.97	0.21
Riddes	Valais	9.7	1.9	33.96	2.92	2.00	1.30	8.80	
Le Guercet/Martigny	Valais	9.6	1.7	83.24	11.68	16.20	4.33	8.85	0.64
Chateauneuf	Valais	9.4	0.6	44.37	1.92	13.20	2.85	8.34	0.78
Sion	Valais	9.4	0.2	37.82	2.90	9.00	2.55	4.63	0.73
Aigle	Valais	8.7	1	22.56	1.59	5.60	0.93	8.79	0.78
Rolle	Valais	8.6	1.8	34.67	2.43	0.00			
Gampel	Valais	8.4	0.1	41.62	1.92	1.60	0.68	5.66	0.76
Glis (dir. Eyholz)	Valais	8.1	-0.3	26.10	1.15	0.40	0.24	8.30	
Montreaux	Midlands	9.4	2.4	35.43	1.01	7.80	0.97	8.83	1.16
Geneva	Midlands	9.3	2	36.33	3.99	9.80	2.89	8.24	1.31
Etzgen	Midlands	8.8	1.2	42.91	2.84	14.60	2.44	6.88	0.74
Zürich-Zollikon	Midlands	8.7	0.9	49.06	1.30	18.00	2.55	6.66	0.35
Schupfart	Midlands	8.5	1.2	32.64	3.32	6.20	2.92	8.75	0.89
Wädenswil	Midlands	8.5	0.7	47.57	2.49	4.80	2.33	10.74	0.23
Breitenhof	Midlands	8.1	0.6	42.39	3.86	3.20	2.52	6.72	0.98
Pfungen	Midlands	8	0.2	37.81	1.90	12.20	2.75	8.81	0.86
Bern-Ostring	Midlands	7.7	0	38.49	2.33	14.20	4.49	6.52	0.77
Auvernier	Jura	8.7	1.6	35.37	2.25	9.00	2.17	8.44	0.86
Courrendin	Jura	8	0.4	34.78	0.76	10.40	2.25	7.68	0.98
Dombresson	Jura	6.7	0.1	43.42	1.01	14.40	4.20	8.50	1.76