# A temperature-based model for predicting the immigration of *Cacopsylla melanoneura* and *C. picta*, vectors of the apple proliferation disease, in South Tyrol, Northern Italy

## (Hemiptera: Psylloidea)

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**Summary:** Insects in temperate regions usually emerge in spring, based on climatic cues. Temperature-based models that predict the first flight onset of pest insects in crop fields are therefore useful tools for agriculture and crop protection. Here, we calibrate and apply such a temperature-based model for *Cacopsylla melanoneura* and *Cacopsylla picta* (Hemiptera - Psyllidae) in South Tyrol, Northern Italy. These psyllid species are the main vectors of apple proliferation, an economically important phytoplasma disease. We found that the temperature associated with the onset (first presence) of the insects in the orchards differs between vectors and between different regions. For the regions Burggrafenamt and Vinschgau, the first adults of *Cacopsylla melanoneura* remigrants mostly occurred in January while first *Cacopsylla picta* remigrants mostly occurred in the orchard between March and April. The presented temperature-based immigration model may be used as a tool to predict the first vector appearance in apple orchards in South Tyrol.

**Keywords:** Apple proliferation, crop protection, flight initiation, pest management, phytoplasma, population dynamics, psyllid, temperature sum model

## 1. Introduction

Insects are ectothermic animals and thus dependent on higher air temperatures for activity. In temperate regions, insects are usually not active in winter and emerge in spring when the temperatures are more favorable. Information about the flight initiation of pest insects are important to anticipate the insect pest movement into the crop fields. The initiation of insect flight responses to a combination of environmental factors, such as availability of food resources, the properties of the organism itself, and temperature (Taylor 1963, Johnson et al. 1992, Perez-Mendoza et al. 2011, Schellhorn et al. 2014). In a recent study on flight initiation using the Rutherglen bug, *Nysius vinitor* Bergroth (Hemiptera: Orsillidae), Moradi-Vajargah and Parry (2017) showed that most of the variation in flight initiation of the insect was related to temperature. Therefore, the flight onset (i. e. the first encounter of pest insect adults in a crop field) can often be predicted based on climatic cues, for example temperature sums (Maixner and Langer 2006). Models based on these cues can be used to support pest management strategies. An example is the web-tool 'Vitimeteo' (Bleyer et al. 2014), which contains a module "VM Schwarzholz", predicting the flight onset of the planthopper *Hyalesthes obsoletus*, the vector of the grapevine phytoplasma disease 'bois noir'.

Apple proliferation (AP) is a phytoplasma disease causing severe economic damage in European apple production areas (Kunze 1989). AP phytoplasma is vectored by two psyllid species, *Cacopsylla melanoneura* (Förster) and *Cacopsylla picta* (Förster) (Hemiptera - Psyllidae)

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(Frisinghelli et al. 2000, Jarausch et al. 2003, Tedeschi et al. 2002). Recently, *C. picta* was found to transovarially transmit AP phytoplasma to its offspring (Mittelberger et al. 2017), and is considered a more effective vector of AP phytoplasma (Baric et al. 2009).

The life cycle of both vectors is summarized by Jarausch and Jarausch (2010). In brief, *C. melanoneura* is univoltine and hibernates in adult stage on overwintering plants, mostly conifers (Lal 1934, Novak and Achtziger 1995, Ossiannilsson 1992, Pizzinat et al. 2011). In South Tyrol, the coniferous forests are mainly restricted to the valley's hillsides. Early in the year, *C. melanoneura* adults (so called "remigrants") migrate to their host plants for oviposition. Being oligophagous, the species uses different Rosaceae species as host plants, for example hawthorn (*Crataegus* spp.) and apple trees (*Malus* spp.). The new generation ("emigrants") then migrates to its overwintering sites in mid-summer (Mayer et al. 2009, Tedeschi et al. 2012, Tedeschi et al. 2002).

*C. picta* is also univoltine, but feeds monophagously on apple trees (Jarausch and Jarausch 2010). The occurrence of *C. picta* in apple orchards is usually timely delayed compared to *C. melanoneura*. *C. picta* remigrates into apple orchards in March/April and the new generation emigrates to the overwintering sites in late summer (Jarausch et al. 2008).

Even though the lifecycle of the AP vectors is relatively well understood, no predictive models for their remigration into orchards are so far available for South Tyrol. The aim of this study was to analyze the relationship between first occurrence of the two AP vectors in apple growing regions in South Tyrol and temperature sums.

## 2. Study sites

The study area included the six main apple growing regions of South Tyrol, Northern Italy (Fig. 1): Located on the South side of the Alps, the landscape of South Tyrol is dominated by valleys at elevations as low as 200 m a.s.l. and mountain peaks reaching 3000 m a.s.l.. The elevations of the surveyed orchards ranged from 200 m a.s.l. in the valleys to 1000 m a.s.l. in the adjacent hill sides.

## 3. Material and Methods

### 3.1 Psyllid vector sampling

Monitoring data on both AP vectors, *C. melanoneura* and *C. picta*, were provided by both the "Laimburg Research Centre" (Fischnaller et al. 2017b) and the South Tyrol advisory council "Südtiroler Beratungsring". 178 orchards were surveyed between 2013 and 2016. Psyllid vectors were collected using yellow sticky traps and the "beating tray"-method (Horton 1999, Muther and Vogt 2003). For the latter, 20 to 200 apple trees, depending on orchard size, were randomly selected for vector sampling. Species identification followed the keys by Burckhardt (2010), Burckhardt and Lauterer (2009) and Ossiannilsson (1992).

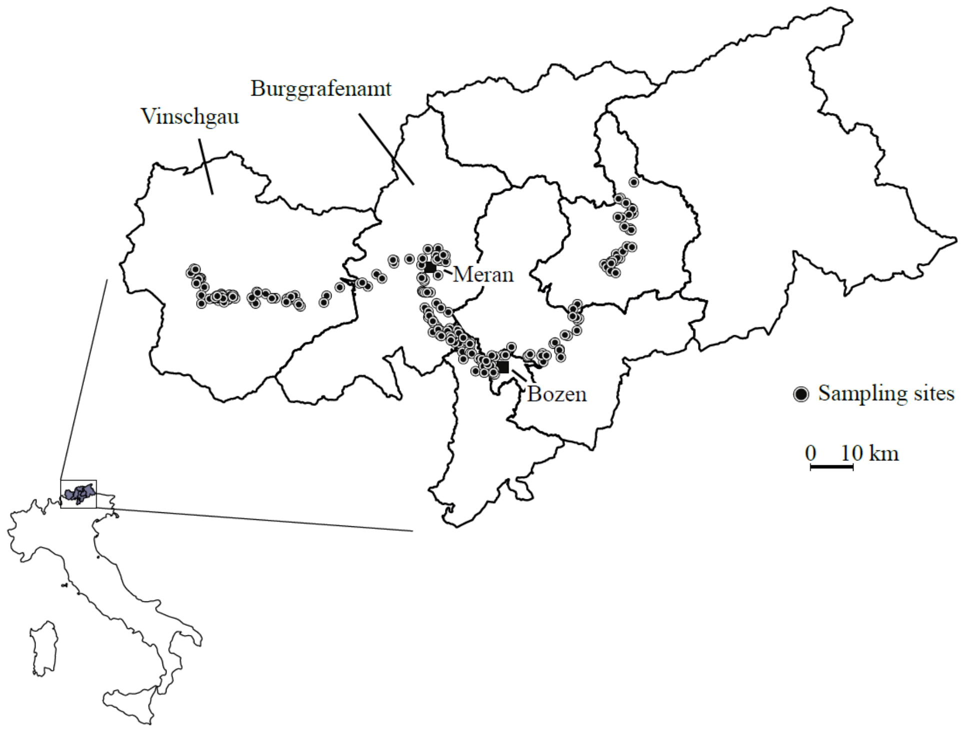



**Fig. 1:** Sampling sites of *Cacopsylla melanoneura* and *Cacopsylla picta* in South Tyrol, Northern Italy.

### 3.2 Weather data

Daily minimum, mean and maximum air temperatures were obtained from weather stations provided by the Hydrographic Office of the Autonomous Province Bozen (http://www.provinz.bz.it/wetter/home.asp, download October 2016): The numbers of weather stations per region were: 1 - Bozen, 7 - Burggrafenamt, 8 - Eisacktal, 5 - Salten-Schlern, 4 - Überetsch-Unterland, 4 - Vinschgau.

### 3.3 Regional temperature-based immigration analysis

Following the methods outlined in Tedeschi et al. (2012), we applied a temperature-based immigration analysis (TempIA) for both AP psyllid vectors. TempIA is based on two indices, a temperature threshold and an immigration index. Psyllid immigration is triggered either when the region-specific temperature threshold or the immigration index of zero is exceeded. In their study, Tedeschi et al. (2012) additionally used the immigration index to estimate the immigration trend into the orchard. This was outside the scope of this study, and therefore, we use the immigration index only as another threshold (fixed at zero) above which we expect AP vector presence in the orchard.

The steps for TempIA calculation were as follows:

(1) Average 10 minute weather data from weather stations to 1 hour temperatures ($\mathbf{T}_{\mathbf{hourly}}$)

(2) Combine beating and yellow trap data for *C. melanoneura* and *C. picta* for remigrants and emigrants

(3) Calculate date of first captured presence per subregion and species (**$a_0$**)
(4) Take the mean of all weather stations' average hourly data within the same subregion (**$^{T}$hourlyregion**)
(5) Calculate the max. temperature (**$T_{0max}$**) within 7 days preceding date of first presence ($a_0$), from $^{T}$hourlyregion
(6) Min. of $T_{0max}$ over all years for the same subregion gives subregional **$T_7$th**
(7) Hourly immigration index (**Ii**) for each region.

The immigration index (Ii) is based on the following equation:

$$Ii = [(T_{7n} - T_{7th}) + ddn] \quad (1.1)$$

with Ii = immigration index; $T_{7n}$ = mean temperature in the 7 days before observation; $T_7$th = highest hourly temperature in the 7 days before observation, and ddn = medium daily number of hours in 7 days before observation with temperatures > T7th.

Finally, we only considered remigrants of *C. melanoneura* and *C. picta* and the apple growing regions "Burggrafenamt" and "Vinschgau" (Fig. 1) for the TempIA model. The reason for this was that there were no sufficient data sets available for the other regions or information about vector absences was missing before the first annual capture. Therefore, insufficient data availability from regions other than "Burggrafenamt" and "Vinschgau" did not allow to draw reliable conclusions about their temperature thresholds, yet.

The R-code for the calculation of the temperature thresholds and immigration indices is provided in Appendix A (electronical).

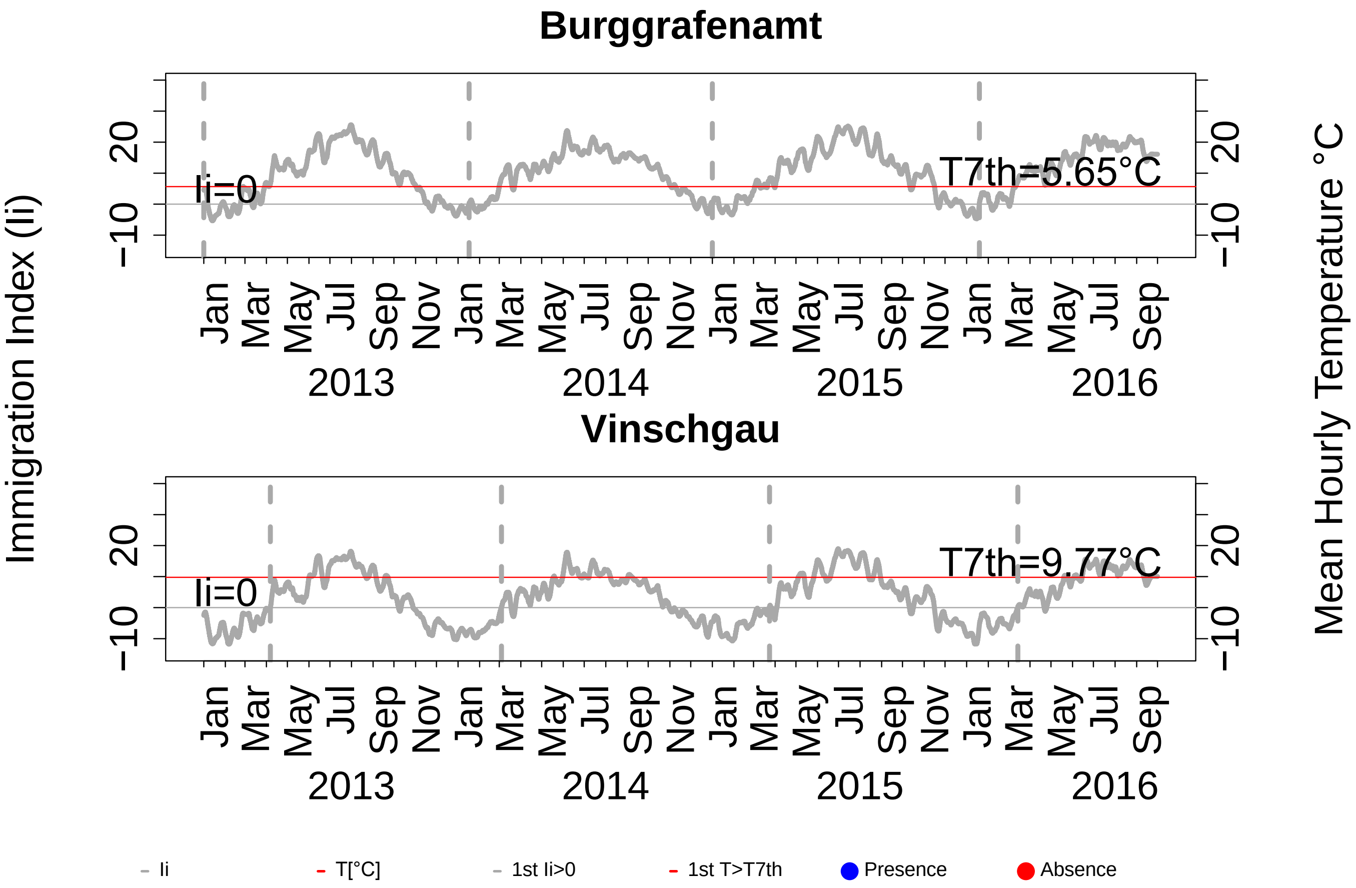
## 4. Results

Mean maximum daily temperatures in 7 days before first occurrence of *C. melanoneura* remigrants were 5.65°C and 9.77°C for the regions Burggrafenamt and Vinschgau (Fig. 2a): Across both regions, the first presence of *C. melanoneura* is mostly predicted to start in January, except for Vinschgau in 2014, where immigration was predicted not before early March.

The TempIA model predicted that the first presences of *C. picta* remigrants in the apple orchards were mostly between March and April (Fig. 2b): The $T_7$th values were 12.73°C and 13.82°C for Burggrafenamt and Vinschgau, respectively. In Burggrafenamt in 2013, 2015 and in Vinschgau in 2015 the temperature threshold was already exceeded in January which disagrees survey observations.

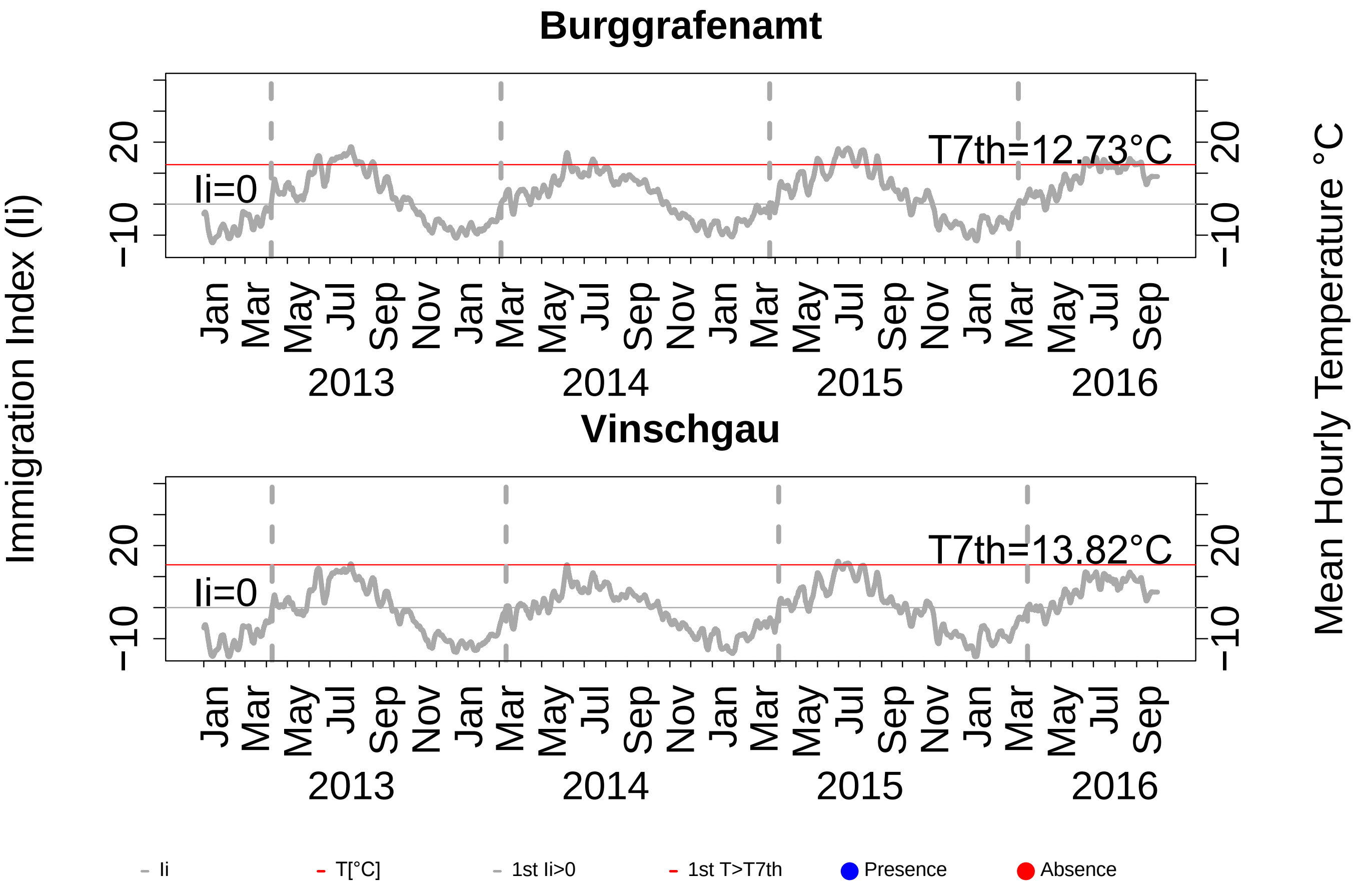
**Fig. 2 (next pages):** Immigration index and temperature thresholds for remigrants of (a) *Cacopsylla melanoneura* and (b) *Cacopsylla picta* by region. The horizontal lines indicate the thresholds for temperature (red, T7th) and immigration index (grey, Ii): The red line is the mean hourly temperature, the grey line is the immigration index. Vertical lines indicate the date when the temperature (red) or the immigration index (grey) thresholds are reached. Red points are species absences and blue points are presences. The insect vector presence in the orchard can be expected either when Ii > 0 or the hourly temperature exceeds.

(a)



Burggrafenamt
Ii=0
T7th=5.65°C
Vinschgau
Ii=0
T7th=9.77°C
Immigration Index (Ii)
Mean Hourly Temperature °C
−10
20
Jan
Mar
May
Jul
Sep
Nov
2013
2014
2015
2016
Ii
T[°C]
1st Ii>0
1st T>T7th
Presence
Absence

(b)


Burggrafenamt
T7th=12.73°C
Ii=0
20
−10
Jan
Mar
May
Jul
Sep
Nov
2013
2014
2015
2016
Vinschgau
T7th=13.82°C
Ii=0
Immigration Index (Ii)
Mean Hourly Temperature °C
Ii
T[°C]
1st Ii>0
1st T>T7th
Presence
Absence

## 5. Discussion

To predict the annual first occurrence of the two AP vectors *C. melanoneura* and *C. picta* in apple orchards following a hibernation period in South Tyrol, Northern Italy, we applied a temperature-based model developed by Tedeschi et al. (2012). The model determines an absolute temperature threshold and an immigration index. The first to be exceeded is accepted as the temperature trigger for vector remigration into the apple orchards. The aim of our analysis was twofold: First, the model allowed us to define a temperature immigration threshold for each region, which can be ecologically interpreted, and second, predictions derived from the model provide an opportunity to farmers to adapt their management decisions to climate fluctuations.

### Immigration-thresholds for *C. melanoneura* and *C. picta*

Our temperature analysis for regions with a sufficient amount of *C. melanoneura* observations (Burggrafenamt, Vinschgau) indicates differences in their estimated temperature thresholds, ranging from 5.65°C to 9.77°C. Tedeschi et al. (2012) calculated the $T_7$th for Trentino to be around 9.5°C, which lies within our calculated threshold range. Fig. 2a shows that the TempIA predictions (indicated by either Ii > 0 or $T_7$th <= hourly temperature) were mostly similar between the years, indicating a potential first occurrence of *C. melanoneura* remigrants in South Tyrolean apple orchards already in January.

*C. picta* capture rates were lower or absent across all apple growing regions in South Tyrol. For regions with sufficient data on *C. picta*, our estimates indicate that *C. picta* begins its immigration into the orchards at higher temperatures than *C. melanoneura* (Fig. 2b). This matches our expectations from the migratory patterns, as well as previous studies, which show that *C. picta*'s immigration starts at a later date with consequently higher ambient temperatures than *C. melanoneura* (Mattedi et al. 2008).

### Limitations of the temperature-based immigration model

The TempIA model assumes that the insect vector presence in the orchard is triggered by a 7-day temperature average. While it is biologically plausible that the insects adjust their emergence to temperature cues, it is unclear if this 7-day air temperature average corresponds well to the true temperature cue that triggers the behavior of the insects. Likewise, it would be possible that the insects respond to other or more complicated triggers, e. g. day-night temperature differences, humidity, or soil temperatures, possibly in connection with other cues or mechanisms, such as a biological clock or timer that is found in many organisms.

Skepticism regarding the 7-day temperature average as sole cue is supported by our model predictions of a first presence of *C. picta* in Burggrafenamt in 2013, 2015 and in Vinschgau in 2015 in January which does not correspond to field observations of this vector (Jarausch et al. 2008, Fischnaller et al. 2017a, Fischnaller et al. 2017b) . In these cases, the immigration index captured more accurately the potential first occurrence in the apple orchard, but of course the reservations named earlier may also be applied to this index. However, discrepancies between actual captures and predicted occurrence based on the temperature threshold may also be explained by data problems. Firstly, the data from weather stations are only an indirect predictor for the temperature on the shelter plants at overwintering sites, and the quality of the correlation may differ from year to year. Currently, no information is available about the exact location and elevation of the overwintering sites of both insect vectors in South

Tyrol, so even if more detailed weather data was available, we would not know which locations are actually relevant. Habitat models including landscape, regional and local environmental factors could be used to identify locations with highest occurrences probabilities (e.g. Panassiti et al. 2013, Panassiti et al. 2017, Sander et al. 2018). According to Tedeschi et al. (2012), especially distance between overwintering site and orchard, geographical layout and air currents may influence psyllid immigration process into apple orchards. On the other hand, if known, temperature measurements are ideally taken directly at the overwintering sites.

Another limitation of the TempIA model includes vector data availability. For example, Fig. 2a shows that the model indicated the first occurrence of *C. melanoneura* in Vinschgau in 2015 in January because the temperature threshold was exceeded in this month. Therefore, to exclude the possibility of undetected presences, vector monitoring of *C. melanoneura* should preferably start in January. Moreover, the accuracy of the thresholds depends on the number of years included in the analysis. Hence, before using the presented model for prediction of the first presence of *C. melanoneura* and *C. picta* in apple orchards, this model must be further improved by a better calibration using more years of survey data that cover a larger sampling period.

## 6. Conclusions

We established a temperature-based immigration analysis for the two AP vectors *C. melanoneura* and *C. picta* in South Tyrol, Northern Italy. Although the analysis indicated that the flight onset is region-specific, we found a general trend that *C. melanoneura* was present in the apple orchard earlier than *C. picta*. The TempIA model indicated that *C. melanoneura* may be present already in January, and *C. picta* assumably in March/April. The temperature threshold ranged from 5.65 to 9.77°C and 12.73 to 13.82°C for *C. melanoneura* and *C. picta*, respectively. However, additional data and further validations are necessary to generate regionally customized, reliable thresholds. Finally, regional-specific immigration starting dates derived from those thresholds can be used to adapt insect vector management strategies.

## 7. Zusammenfassung

Ein temperaturbasiertes Modell zur Immigration von *Cacopsylla melanoneura* und *Cacopsylla picta*, Vektoren der Apfeltriebsuchtkrankheit, in Südtirol, Norditalien (Hemiptera, Psylloidae). – Die Aktivität von Insekten ist stark temperaturabhängig. Temperatursummenmodelle, welche den Beginn der Flugaktivität von Schadinsekten voraussagen, sind daher sinnvolle Instrumente im Pflanzenschutz. In dieser Untersuchung wenden wir ein auf Temperatursummen basierendes Modell auf *Cacopsylla melanoneura* und *Cacopsylla picta* (Hemiptera - Psyllidae) in Südtirol (Norditalien) an. Beide Psyllidenarten sind die Hauptüberträger der Apfeltriebsuchtkrankheit (AP), einer ökonomisch bedeutenden Phytoplasmose. Wir fanden heraus, dass sich das erste Vorkommen in den Apfelanlagen zwischen den Vektoren und den einzelnen Regionen Südtirols unterscheidet. Für die Regionen Burggrafenamt und Vinschgau deutete das Modell darauf hin, dass die ersten adulten Tiere von *Cacopsylla melanoneura* (Remigranten) bereits im Januar in Apfelanlagen vorkommen können, während ein erstes Auftreten von *Cacopsylla picta* (Remigranten) im März/April am wahrscheinlichsten ist. Zusammenfassend kann man sagen, dass der Flugbeginn abhängig von der Vektorart, dem

Jahr und der jeweiligen Region ist. Nach weiterer Validierung könnte das vorgestellte Temperatursummenmodell zur Vorhersage des ersten Auftretens beider AP-Vektoren in Apfelanlagen in Südtirol genutzt werden.

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## Appendix

Appendix A: R-code for the temperature immigration analysis:
https://github.com/berndpanassiti/Temperature-based_immigration_analysis/blob/master/r-code/TempIA.md

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