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Evaluation of the biological activity of granulovirus isolates from *Tuta absoluta* (*Tuab*GV) and *Phthorimaea operculella* (*Phop*GV) in its primary and secondary host *Tuta absoluta* (Meyrick) (Lep., Gelechiidae)

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LIST OF ACRONYMS

ANOVA	Analysis of variance
Bt	*Bacillus thuringiensis* Berliner
BV	Budded virions
CIP	Centro Internacional de la Papa (International Potato Center)
DF	Degrees of freedom
DNA	Desoxyribonucleic acid
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
GLM	Generalized linear model
GV	Granulovirus
HR	Relative humidity
IPM	Integrated Pest Management
IRAC	Insecticide Resistance Action Commitee
ISO	International Organization for Standardization
kbp	kilo base pairs
LC	Lethal concentration
MNPV	NPV with multiple nucleocapsids
NPV	Nucleopolyhedrovirus
ODV	Occlusion-derived virions
OB	Occlusion body
*Phop*GV	*Phthorimaea operculella* Granulovirus
SD	Standard deviation
SNPV	NPV with single nucleocapsid
*Tuab*GV	*Tuta absoluta* Granulovirus
UV	Ultraviolet radiation

1 ABSTRACT

The tomato leafminer, *Tuta absoluta* (Meyrick), is an insect pest of worldwide importance. By attacking primarily tomato (*Solanum lycopersicum* L.), but also potato (*Solanum tuberosum* L.) and various other crops, it reduces yield and income of small-scale subsistence farmers, as well as of large-scale tomato producers in South America, Africa, Europe and the Middle East.
In this study five isolates of the *Phthorimaea operculella* granulovirus (*Phop*GV) from different regions primarily from Peru (Cuzco, Huancayo, Huaraz, La Molina) and Chile were tested together with two isolates of the *Tuta absoluta* granulovirus (*Tuab*GV) from Peru (La Molina and St. Rita (Arequipa)) to identify the most effective isolates for a potential development as biological insecticide against *T. absoluta*. The efficacy of all seven isolates against *T. absoluta* was examined in an egg dip bioassay at five concentrations (2.50E+05 OBs/ml, 5.00E+06 OBs/ml, 7.50E+07 OBs/ml, 3.00E+08 OBs/ml, and 6.15E+08 OBs/ml).
As a result, *Phop*GV Huancayo, *Tuab*GV La Molina, and *Tuab*GV St. Rita (Arequipa) were considered most promising, showing the best efficacies. A probit analysis resulted in LC_{50}, LC_{95}, and LC_{99} values of 4.27E+08 - 2.05E+09 OBs/ml, 1.95E+09 - 9.56E+09 OBs/ml, and 2.23E+09 - 1.27E+10 OBs/ml, respectively. These mathematically derived lethal concentrations led to the conclusion that a successful control may require higher virus concentrations than used in this study.

Two of the promising isolates (*Phop*GV Huancayo and *Tuab*GV La Molina) were examined further. The efficacy of these selected isolates against the four larval instars of *T. absoluta*, as well as the persistence of the virus over seven days were examined on virus-treated tomato plants under laboratory conditions. The results led to the assumption that the susceptibility of the larvae towards the GV was not affected by larval age. Furthermore, the examination of the virus stability revealed no decrease of biological activity for both isolates over a timespan of seven days, which indicates a high persistence of the virus.

In summary, the findings suggest *Phop*GV as well as *Tuab*GV as potential biological insecticide against *T. absoluta* and furthermore three particularly promising isolates could be identified. The findings gained in this study may contribute to the development of a sustainable control strategy of *T. absoluta*.

2 ZUSAMMENFASSUNG

Die Tomatenminiermotte, *Tuta absoluta* (Meyrick), ist ein Schädling von weltweiter Bedeutung. Der Befall von Tomaten (*Solanum lycopersicum* L.), aber auch Kartoffeln (*Solanum tuberosum* L.) und anderen Nutzpflanzen, führt zu Ertrags- und Einkommenseinbußen sowohl bei kleinbäuerlichen Betrieben, als auch in der industrialisierten Landwirtschaft in Südamerika, Afrika, Europa und dem Mittleren Osten.
In der vorliegenden Studie wurden fünf Isolate des *Phthorimaea operculella* Granulovirus (*Phop*GV) vornehmlich aus Peru (Cuzco, Huancayo, Huaraz, La Molina) und Chile zusammen mit zwei Isolaten des *Tuta absoluta* Granulovirus (*Tuab*GV) aus Peru (La Molina und St. Rita (Arequipa)) getestet. Ziel war es, die effizientesten Isolate zu identifizieren, die potentiell zur Entwicklung eines biologischen Insektizids gegen *T. absoluta* geeignet sind. Alle sieben Isolate wurden in einem Bioassay in fünf verschiedenen Konzentrationen (2.50E+05 OBs/ml, 5.00E+06 OBs/ml, 7.50E+07 OBs/ml, 3.00E+08 OBs/ml und 6.15E+08 OBs/ml) auf ihre Wirksamkeit gegen *T. absoluta* getestet. Drei der getesteten Isolate, nämlich *Phop*GV Huancayo, *Tuab*GV La Molina und *Tuab*GV St. Rita (Arequipa), wiesen insgesamt die höchsten Wirkungsgrade auf. Bei der anschließenden Probit-Analyse wurden LC_{50}, LC_{95} und LC_{99} Werte zwischen 4.27E+08 und 2.05E+09 OBs/ml, 1.95E+09 und 9.56E+09 OBs/ml, beziehungsweise 2.23E+09 und 1.27E+10 OBs/ml, berechnet. Für die erfolgreiche Bekämpfung des Schädlings werden somit vermutlich höhere Konzentrationen, als die in dieser Studie gewählten, benötigt.

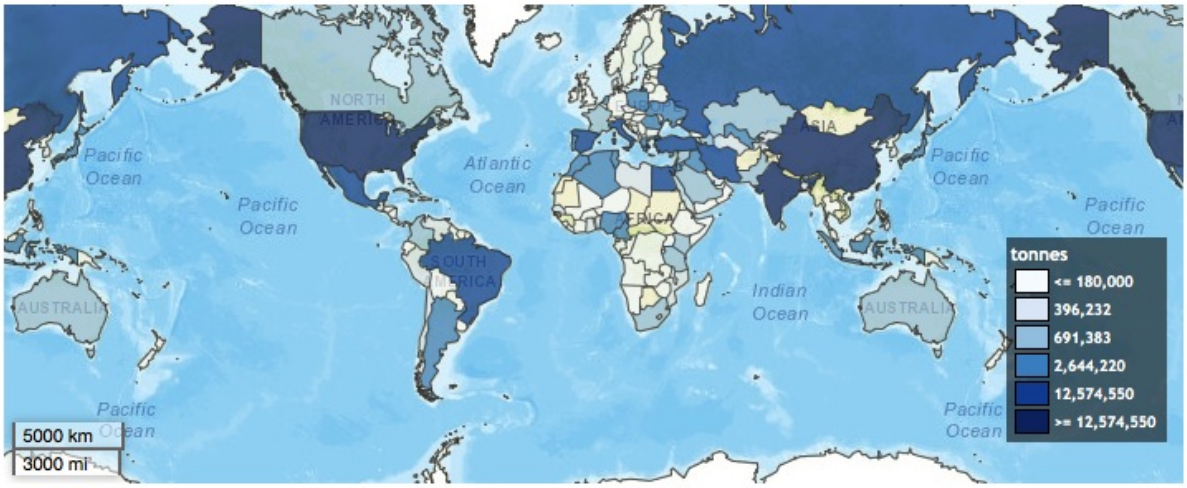
Anhand der zwei aussichtsreichen Isolate *Phop*GV Huancayo und *Tuab*GV La Molina wurden weitergehende Untersuchungen durchgeführt. Die Anfälligkeit der vier Larvenstadien gegenüber der Virusbehandlung, sowie die Stabilität des Virus über einen Zeitraum von sieben Tagen, wurden auf behandelten Tomatenpflanzen unter Laborbedingungen getestet. Dabei wurde eine gleichbleibend hohe Anfälligkeit gegenüber den Granuloviren unabhängig vom behandelten Larvenstadium festgestellt. Ferner zeichneten sich beide Virusisolate durch eine hohe Stabilität aus, die sich durch eine unverändert hohe biologische Aktivität über einen Zeitraum von sieben Tagen nach Applikation, ergab.

Die vorliegende Studie belegt die Wirksamkeit von *Phop*GV sowie *Tuab*GV als potenzielle biologische Insektizide gegen *T. absoluta*. Drei Isolate haben sich hierbei als vielversprechend erwiesen und könnten zur Entwicklung einer nachhaltigen Bekämpfungsstrategie von *T. absoluta* beitragen.

3 INTRODUCTION

3.1 Tomato production worldwide

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world. The tomato ranks on the 11th place of the most produced crops worldwide (FAO 2013c). In the past 20 years, global production more than doubled, from 77.90 million tons/year in 1993 to 163.43 million tons/year in 2013 (FAO 2013a). Top 10 tomato producing countries are China, India, USA, Turkey, Egypt, Iran, Italy, Brazil, Spain, and Mexico (FAO 2013d).
In South America in the year of 2013 tomatoes were produced on an area of 131 398 hectares (FAO 2013b) and yielded 6.7 million tons, ranking on the 14th place of the most produced crops on this continent (FAO 2013c). The gross production value of South American tomatoes was US$ 7250.24 million.



(FAO 2013a)

Fig. 1: Worldwide tomato production in 2013.

Vegetable crops such as tomatoes are higher-priced than basic food crops, resulting in a higher income per hectare for tomato growing farmers (Villareal 1980). Based on economic value, tomatoes rank on the 10th place among the crops with the highest value worldwide (FAO 2013c).
Tomatoes are grown worldwide at various latitudes (Fig. 1). Successful production is possible under a wide range of environmental conditions, at different altitudes, and under various cultivation methods (Villareal 1980).
Tomato production is labour intensive and can be performed in large scale industrialized farming environments as well as in small homegardens. Large farms can provide employment opportunities in rural areas and help increasing income for local workers. Furthermore the up- and downstream sector, such as seedling production, packaging, processing, and sale create additional jobs also in urban areas (Villareal 1980).

Apart from generating income and employment, tomatoes are valuable with regard to nutritional aspects. Carotenoids and phenolics have antioxidant activity and tomatoes serve as major vitamin C source (Chetelat 2014). As an important source of minerals and vitamins, tomato consumption contributes to an improved nutrition and well being (Villareal 1980).

3.2 Insect pests in tomato production

Various biotic and abiotic factors affect tomato production. In tropical cropping systems insect pests are considered the most important biotic constraints of tomato (Kumra & Poehling 2010). Accordingly, most significant tomato pests are from the class Insecta, though some species of mites (Acari) form part of the most important tomato pests, as well (Schuster et al. 2014). The majority of pest species are not specific to tomatoes, but a large number of polyphagous herbivores are known to feed on tomato plants. About 100-200 pest species, predominantly of the families Lepidoptera, Hemiptera, Thysanoptera, and Diptera, have been observed attacking tomato plants worldwide (Kumra & Poehling 2010).
In the tropics a high diversity of species and favourable climatic conditions promote insect development and reproduction rates, resulting in high infestation pressure (Kumra & Poehling 2010).
A large group of pest insects comprise the larvae of lepidopteran insects, such as the tomato fruitworm (*Helicoverpa zea* Boddie), the tomato pinworm (*Keiferia lycpersicella* Busck), cutworms, hornworms (*Manduca* spp.), and several species of armyworms (*Spodoptera* spp.). The caterpillars feed on foliar parts and fruits, often chewing holes or boring mines into the fruit tissue (Schuster et al. 2014). One of these lepidopteran pest species is the tomato leafminer (*Tuta absoluta*).

3.2.1 Tomato leafminer *Tuta absoluta*

The tomato leafminer was described by Meyrick for the first time in 1917 as *Phthorimaea absoluta*, based on an individual found in Huancayo, Peru (Povolny 1975).

Taxonomy

The taxonomic assignment has undergone various changes in the past. Therefore scientific synonyms for *T. absoluta* are *Gnorimoschema absoluta* Clarke (1962), *Scrobipalpula absoluta* Povolny (1964), and *Scrobipalpuloides absoluta* Povolny (1987). Povolny's description from 1994 as *T. absoluta,* is currently the correct name of the species (EPPO 2005; CABI 2015).

Taxonomic classification of *T. absoluta* (Meyrick):

Domain: Eukaryota
Kingdom: Metazoa
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Gelechiidae
Genus: *Tuta*
Species: *Tuta absoluta*

(CABI 2015)

Common English names are Tomato leafminer, Tomato borer, South American tomato moth, and South American tomato pinworm. In Spanish and Portuguese language areas *T. absoluta* is called Polilla del tomate, Polilla perforadora, Cogollero del tomate, Gusano minador del tomate, Minador de hojas y tallos de la papa or Traça-do-tomateiro (EPPO 2005).

Morphology and Ecology

Adult moths are 7-10 mm long, have narrow wings and long, filiform antennae. Their body is of silver to grey colour with black spots on the anterior wings. Females have a wingspan of about 10.7 mm, whereas males are usually a bit smaller with a wingspan of approximately 10.1 mm. The female abdomen is wider and heavier than the male abdomen (Fig. 2) (Vargas 1970; EPPO 2005; Urbaneja et al. 2013).

T. absoluta females lay single eggs on the aerial parts of the host plant, mainly on the underside of the leaves (EPPO 2005). The eggs are of a creamy white colour, turning yellow-orange over time (Fig. 3). They have a cylindrical shape and measure approximately 0.4 mm in length and 0.2 mm in diameter (Vargas 1970; Urbaneja et al. 2013).

Fig. 2: Adult female (left) and male (right) of *T. absoluta*.

Fig. 3: *T. absoluta* eggs.

4-5 days after oviposition the eggs darken right before the larvae hatch (EPPO 2005). After hatching, the larvae remain on the leaf surface for approximately 82 minutes, before they enter the leaf tissue (Cuthbertson et al. 2013).
The following larval development comprises four instars. First instar larvae are creamy coloured with a dark head and a length of about 0.9 - 1.6 mm. During their development, their colour changes to a light green, due to the ingested food (Fig. 4) (Vargas 1970; EPPO 2005; Urbaneja et al. 2013). Throughout the larval stage, the larvae turn more and more greenish in colour. The second instar larvae are about 2.8 mm long and the third instar reaches about 4.7 mm in length (Vargas 1970; Urbaneja et al. 2013). Forth instar larvae are approximately 8.0 mm long and have a light pink shade (Urbaneja et al. 2013). In advance of the moulting at the end of each larval instar, the larval skin turns whitish (Vargas 1970).
At 25-26°C and 60-74.7% humidity, the first instar takes 2.49 – 3.6 days, the second instar takes 2.32 – 3.13 days, the third 2.52 – 3.24 days, while the fourth instar takes 2.71 – 3.79 days (Vargas 1970; Erdogan & Babaroglu 2014; Çekin & Yasar 2015). After a total larval development time of 11-20 days (depending on the environmental conditions, especially the temperature), larvae stop food uptake and enter the pupal stage (Estay 2000; EPPO 2005; Çekin & Yasar 2015).

Pupae are cylindrically shaped and measure approximately 4.3 mm in length and ca. 1.1 mm in diameter. Their green colour turns into a dark brown over the 7-12 days of the pupal development (Fig. 5) (Vargas 1970; Estay 2000; EPPO 2005; Urbaneja et al. 2013).
Usually larvae pupate in the soil, but occasionally, pupae can also be found on the leaves and inside the feeding galleries. If larvae pupate outside the soil, they build a white silk cocoon to protect the pupa (EPPO 2005; Urbaneja et al. 2013).

Fig. 4: *T. absoluta* larva (second instar).

Fig. 5: Early (above) and late (bottom) pupae stage of *T. absoluta*.

Adult longevity depends on several factors. Vargas (1970) discovered that the female moths live longer than the males and unmated moths have a slightly longer lifespan than mated individuals. His results showed that mated males lived on average for 6.6 days, while mated females lived for

10.4 days. If the moths stayed unmated, they lived for 7.2 (males) and 15.2 (females) days, respectively. However, Lee et al. (2014) found no significant differences in the longevity between the sexes.
Females as well as males are able to mate multiple times, but only once a day, usually in the morning hours (Lee et al. 2014). Caparros Megido et al. (2012) and Abbes & Chermiti (2014) found populations of *T. absoluta*, which are able to reproduce parthenogenetically, even though other authors could not confirm those findings so far (Lee et al. 2014).
Adult moths disperse by flight. They are nocturnal with a morning-crepuscular activity, usually hiding between the leaves during the day (EPPO 2005; Urbaneja et al. 2013).
T. absoluta is able to overwinter as an egg, pupa or adult (EPPO 2005).

Temperature-dependent development

T. absoluta is a multivoltine pest with high reproductive potential of up to 10-12 generations per year (EPPO 2005; Desneux et al. 2010; Urbaneja et al. 2013). In the face of global warming it is likely that *T. absoluta* is going to develop even faster and produce more generations per year in the near future (Bale et al. 2002).
Female moths lay about 40-50 eggs per oviposition period, which results in about 260 eggs during their lifetime (Estay 2000; EPPO 2005). The highest ovipostition activity has been observed in the first 2-3 days of the female lifespan, before it decreases rapidly over their remaining lifetime (Lee et al. 2014). The complete lifecycle includes the four stages egg, larva, pupa, and adult (Urbaneja et al. 2013). The duration of the biological cycle depends largely on environmental conditions, especially the temperature. Trials in Chile showed that the full completion of the cycle takes 76.3 days at 14°C, whereas it can be completed in 23.8 days at 27.1 °C (Barrientos et al. 1998). Çekin & Yasar (2015) found a total development time of 22.88-24.5 days at 25°C.
The temperature threshold for the development from egg to adult was estimated at 8.14°C (Barrientos et al. 1998).

Feeding preferences and symptoms of infestation

T. absoluta larvae attack their host plant in any developmental stage. The freshly hatched larvae feed preferably on apical buds, flowers or young fruits, but they also penetrate leaves, stems or ripening fruits. During its development an individual larva might attack several tissue types. When feeding, the larvae create mines in the fruits and stems, as well as galleries in the leaf tissue by feeding on the mesophyll while leaving the epidermis intact (EPPO 2005; Urbaneja et al. 2013). The galleries and mines may become necrotic and serve as an entrance for secondary plant pathogens (Vargas 1970; EPPO 2005). Feeding activity on the flowers stops the fecundation of the host plant (Urbaneja et al. 2013). Infestation is visible due to the black frass of the larvae on infested plants (EPPO 2005).

Host plants

The primary host of the tomato leafminer is the tomato plant, yet a development on other cultivated and wild plants is possible. Another crop targeted by *T. absoluta* is the potato plant (*Solanum tuberosum* L.). Besides, *T. absoluta* was also found on *Solanum melongena* L. (aubergine), *Solanum muricatum* Aiton (melon pear), *Capsicum annuum* L. (pepper), *Nicotiana tabacum* L. (tobacco), *Nicotiana glauca* Graham (tree tobacco), *Physalis philadelphica* Lamarck (physalis), and *Physalis angulata* L. (cutleaf groundcherry). Other alternative hosts are wild Solanaceous plants, such as *Solanum nigrum* L., *Solanum lyratum* Thunberg, *Solanum habrochaites* S. Knapp & D.M. Spooner, *Solanum elaeagnifolium* Cavanilles, *Solanum dubium* Fresen, *Solanum woronowii* Pojark, *Brugmansia arborea* (L.) Lagerhein, *Datura stramonium* L., *Datura ferox* L., and *Lycium chilense* Miers ex Bertero.

Hosts from other plant families have been reported as well, such as *Citrullus lanatus* (Thunb.) Matsum. & Nakai (watermelon, Cucurbitaceae), *Amaranthus viridis* L., *Amaranthus spinosus* L. (Amaranthaceae), *Sonchus oleraceus* L., *Xanthium strumarium* L., *Xanthium brasilicum* Vellozo (Asteraceae), *Sinapis arvanesis* L. (Brassicaceae), *Convolvulus arvensis* L. (Convolvulaceae), *Medicago sativa* L., *Phaseolus vulgaris* L. (Fabaceae), *Sorghum halepense* (L.) Pers. (Poaceae), and *Jatropha curcas* L. (Euphorbiaceae) (Vargas 1970; Larraín S. 1987; Estay 2000; CABI 2015; Mohamed et al. 2015; EPPO 2016b).

Importance as a pest

T. absoluta is considered one of the most devastating pests of tomato crops (Estay 2000; Desneux et al. 2010; Zlof & Suffert 2012). Especially in South America, but also in all newly infested areas, it is considered a serious threat of tomato cropping and a key pest of tomatoes produced in the field, as well as under protected conditions (EPPO 2005; Urbaneja et al. 2013).

Furthermore, it is considered a serious threat to potato cropping (Herrera Arangúena 1963; Vargas 1970; Campos 1976). Currently *T. absoluta* is listed on the EPPO A2 list of pests recommended for regulation as quarantine pests (version 2015-09).

The galleries in leaves and stems alter general development of the plants by diminishing the photosynthetic capacity and therefore reducing the yields. Injuries directly made to the fruits reduce the yield as well as the fruit quality severely, decreasing their commercial value (EPPO 2005; Urbaneja et al. 2013). Without control measures, yield loss and reduction of quality by direct feeding and secondary pathogens, can reach up to 100% in tomato crops (EPPO 2005; Urbaneja et al. 2012). In newly infested areas, the application of insecticides increases, resulting in increasing production costs for the farmers (Desneux et al. 2011).

For those reasons, infestation with *T. absoluta* can be a threat to the economy of tomato exporting countries. Especially China, India, United States of America, Turkey, and Egypt, the top five tomato exporting countries in 2013, could experience severe losses (Desneux et al. 2011;

FAO 2013d). In 2011, Desneux et al. estimated that more than 80% of the tomato cropped surface and tomato production worldwide, are threatened by *T. absoluta* infestation.

T. absoluta in potato

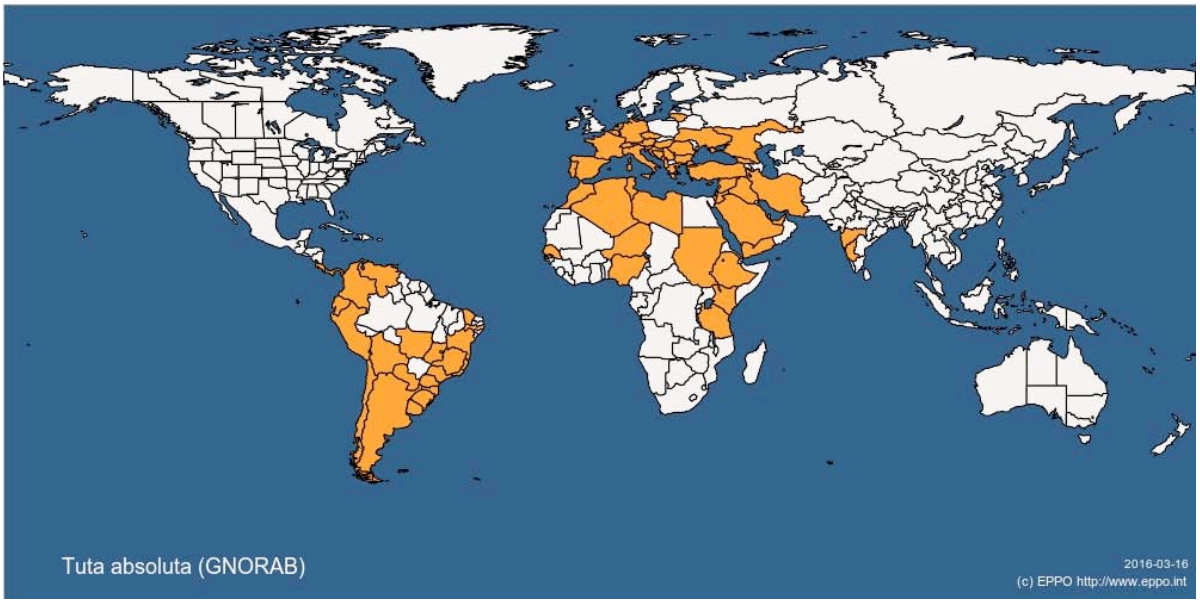
Potatoes rank on the 6th place of the most produced crops worldwide, with a total production of 374.46 million tons/year (FAO 2013c). They are considered the third most important food crop in terms of human consumption (Haase et al. 2015). Top 10 potato producing countries in 2013 were China, India, Russia, Ukraine, United States of America, Germany, Bangladesh, Poland, France, and the Netherlands (FAO 2013d).
More than 4000 different potato varieties grow in the Andean region. Especially for small farmers potatoes are, together with a wide range of other solanaceous crops, important basic foodstuff (Haase et al. 2015).

Herrera Arangúena described *T. absoluta* already in 1963 as a problematic pest in Peruvian potato fields. Though Pereyra & Sánchez (2006) found tomato to be a more suitable host plant for the tomato leafminer, they showed at the same time that under favourable conditions a development on potato is also possible. Other scientists have even come to the conclusion that potato has a similar suitability for *T. absoluta* development like tomato (Caparros Megido et al. 2013). Studies reporting of *T. absoluta* as a potato pest are contradictory about which parts of the potato plant are targeted. Some state that the larvae only attack the aerial parts and do not directly harm the tubers, while others report feeding damage on the tubers as well (Vargas 1970; Pereyra & Sánchez 2006; Mohamed et al. 2012; Zlof & Suffert 2012; Urbaneja et al. 2013).
Especially in Peru, *T. absoluta* is a pest with significant damage in potato production (Cisneros & Mujica 1998; Pereyra & Sánchez 2006). However, infestation of potato crops have not only been reported from South America, but also from other parts of the world, e.g. Sudan and Turkey (Herrera Arangúena 1963; Campos G 1976; International Potato Center 1996; Unlu 2009; Mohamed et al. 2012).
With potato being an important basic food crop, *T. absoluta* attacking potato plants is a potential threat to worldwide potato production and food security.

Worldwide distribution of *T. absoluta*

T. absoluta is originally native in South America (Desneux et al. 2010; Urbaneja et al. 2012). It spread throughout South America and since was found in Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela (Barrientos et al. 1998; EPPO 2005). Since its first detection in Spain in 2006, it invaded other countries in Europe, the Mediterranean basin, Northern Africa, and the Middle East (Desneux et al. 2010; Desneux et al. 2011).

Currently the pest is present in many South American countries and even has reached Costa Rica. In Africa it is now present from Northern Africa down to Tanzania in the East and Senegal and Nigeria in the West. In Europe, it spread to the Northwest up to Lithuania and eastwards to the Middle East and up to India (Fig. 6). EPPO compiled a list of all countries, from where *T. absoluta* infestations were reported (EPPO 2016a). (For the entire list of countries with *T. absoluta* occurrence see Table A1 in the annex).



(EPPO 2016a)

Fig. 6: Countries of current distribution of *T. absoluta*.

Analysis of the genetic variability of *T. absoluta* populations from South America and the Mediterranean Basin indicated that the invading populations in Europe, Africa and the Middle East originate from a single introduction from a Chilean line (Guillemaud et al. 2015).

T. absoluta is found up to high altitudes (e.g. the Andean region). The first description by Meyrick was given on the basis of a holotype, found at an altitude of about 3500 m, in Huancayo, Peru (Povolny 1975).

With its rapid geographical spread, *T. absoluta* has developed from a South American pest into a worldwide threat to tomato production (Desneux et al. 2010; Desneux et al. 2011). Although the history of its spread as well as pheromone trap trials indicate that *T. absoluta* can disperse actively without human help, the rapid dispersal seems to be linked to the global trade of tomato fruits (Desneux et al. 2011; Urbaneja et al. 2013). Further expansion of *T. absoluta* populations in other areas of the world seems likely (Urbaneja et al. 2013).

Control methods

The first step to determine the occurrence and density of a pest in the field is to monitor the population level with the help of pheromone traps. A synthetic sex pheromone was developed:

(3Z,8Z,11Z)-3,8,11-tetradecatrienyl acetate, which is identical to the natural pheromone and effectively attracts *T. absoluta* males (Attygalle et al. 1996). It is highly species-specific and is even successful at very low population densities (EPPO 2005; Urbaneja et al. 2013).
Until 2013, there was no standard economic threshold established for the control of *T. absoluta* (Urbaneja et al. 2013). In South America an economic injury level of 45±19.5 adults/trap/day was defined by Benvenga et al. (2007). Under greenhouse conditions, a density of 26 larvae/plant were found to cause a considerable decrease in yield (Cely et al. 2010).
Another monitoring method is a visual supervision by plant sampling. In comparison though it is considered more difficult and time consuming than monitoring with pheromone traps (Urbaneja et al. 2013). Recently an action threshold of 36, 43, and 60% infested leaves in cultivars with big, medium and small fruits, respectively, was suggested in Italy. This corresponds to an economic threshold of 1% damaged fruits after two weeks (Cocco et al. 2015).

Chemical control

Chemical control has been the dominant control strategy for *T. absoluta* management (Lietti et al. 2005; González-Cabrera et al. 2011; Lo Bue et al. 2012). Several treatments per growing season are required to control *T. absoluta* successfully (EPPO 2005). In areas with high infestation pressure, growers have to apply up to 25 treatments per season (Zlof & Suffert 2012).
A wide range of insecticides for the control of *T. absoluta* control has been registered (Table 1).

Table 1: List of chemical insecticides, which are registered for the control of *T. absoluta*.

CHEMICAL CLASS	ISO COMMON NAMES
Organophosphates	Chlorpyrifos, Methamidophos
Pyrethroids	Bifenthrin, Cyfluthrin, *beta*-Cyfluthrin, *gamma*-Cyhalothrin, *lambda*-Cyhalothrin, Cypermethrin, *alpha*-Cypermethrin, *beta*-Cypermethrin, *zeta*-Cypermethrin, Delthamethrin, Esfenvalerate, Etofenprox, *tau*-Fluvalinate, Fenpropathrin, Permethrin
Spinosyns	Spinetoram, Spinosad
Avermectins, Milbemycins	Abamectin, Emamectin benzoate
Pyrroles	Chlorfenapyr
Benzoylureas	Diflubenzuron, Flufenoxuron, Lufenuron, Novaluron, Noviflumuron, Teflubenzuron, Triflumuron
Diacylhydrazines	Chromafenozide, Methoxyfenozide, Tebufenozide
Oxadiazine	Indoxacarb
Semi-carbazone	Metaflumizone
Diamides	Chlorantraniliprole, Flubendiamide
Nereistoxin analogues	Cartap

Adapted from IRAC (2011). Resistant *T. absoluta* populations have been found against substances from chemical classes typed in bold letters.

At the first outbreaks of *T. absoluta* in South America, organophospates were mainly used to control the pest. Those were in the 1970s replaced by pyrethroids. In the 1980s cartap, pyrethroids, and thiocyclam provided efficient control of *T. absoluta* (Lietti et al. 2005). During the 1990s new substances such as acylurea insect growth regulators, spinosad (spinosyns), abamectin (avermectins), tebufenozide (diacyhydazines), and chlorfenapyr (pyrroles) were introduced to the market (Desneux et al. 2010). Additionally, new pyrethroids were tested and found to be efficient pesticides in Brazil, while flubendiamide and chlorantranilipole (diamides), emamectin benzoate (avermectins), and spinosad (spinosyns) have provided efficient control of *T. absoluta* in Europe (Silvério et al. 2009; Roditakis et al. 2013).

Development of insecticide resistances

Since the 1980s, a decreasing efficacy of chemical plant protection agents in tomatoes was observed (EPPO 2005). Nowadays, resistances are reported from several countries; in Latin America resistances to organophosphates, pyrethroids, chloride channel activators, and benzoylureas are widespread (IRAC 2011). In 1997, a study by Salazar & Araya reported resistances against deltamethrin, esfenvalerat, and *lambda*-cyhalothrin (pyrethroids), as well as mevinfos and metamidofos (organophosphates) in Chile. In Argentina, populations with resistances against abamectin (avermectins) and deltamethrin (pyrethroids) were found (Lietti et al. 2005). In Brazil resistances against abamectin, cartap (nereistoxin analogues), permethrin (pyrethroids), and methamidophos (organophosphates) were detected (Siqueira et al. 2000). A few years later Silva et al. (2011) could not confirm the resistance of permethrin, but found moderate resistances against indoxacarb (oxadiazine) and moderate to high resistances against the chitin synthesis inhibitors diflubenzuron, teflubenzuron, and friflumuron in Brazil. Additionally, control failures of bifenthrin and permethrin (pyrethroids), as well as *Bt* were reported (Silva et al. 2011).

In Europe the pyrethroid cypermethrin is insufficient in controlling *T. absoluta*. Furthermore, potential control failures were detected for metaflumizone (semi-carbazone) and chlorpyriphos (organophospate) (Roditakis et al. 2013).

Especially in tropical countries resistances against chemical pesticides evolve faster in insect populations due to their capability to undergo multiple generations per year (Cunningham 1995).

Biological control

In the area of its origin, many antagonists of *T. absoluta* are described. Meanwhile some indigenous natural enemies have also been found in the Mediterranean region (Urbaneja et al. 2013). Desneux et al. (2010) compiled a list of parasitoids, all from the taxonomic order Hymenoptera, associated with *T. absoluta* occurring in South America. Members of the families Encytridae and Eupelmidae were found to parasitize the leafminer eggs. Various species of Trichogrammatidae are egg parasitoids, as well (Desneux et al. 2010). Though, for successful control, *Trichogramma* parasitoids have to be released several times inundatively as an effective establishment is problematic (Chailleux et al. 2013). According to Miranda et al. (1998), the

larval stage is the most critical one with the highest natural mortality. Though the mortality due to parasitism is much lower than due to predation. Species of the families Bethylidae, Braconidae, Eulophidae, Ichneumonidae, and Tachinidae are parasitizing the larval stage (Desneux et al. 2010).
Only a few species from the families Braconidae, Chalcididae, and Eulophidae were identified as parasitoids of the pupal stage. There are no parasitoids known that attack the adult moths. A list of parasitoids occurring in the Mediterranean region, compiled by Zappalà et al. (2013), included additionally species of the family Pteromalidae (order Hymenoptera). Temperature as well as the appropriate parasitoid strain are essential for a successful control with parasitoids (Pratissoli & Parra 2000; Pratissoli & Parra 2001).

Urbaneja et al. (2013) listed predators of *T. absoluta* from South America as well as from the Mediterranean region. It includes species of the families Phytoseidae and Pyemotidae (order Acari), Araneidae (Araneae), Cochinellidae (Coleoptera), Anthocoridae, Miridae, Pentatomidae, Berytidae and Nabidae (Hemiptera), Vespidae (Hymenoptera), Chrysopidae (Neuroptera), and Phlaeothripidae (Thysanoptera). A list of predators found in the Mediterranean region, compiled by Zappalà et al. (2013), included in addition the families Sphecidae and Formicidae (order Hymenoptera). The predators found in Europe and North Africa so far prey only on eggs and young larvae, achieving an effective pest control only for a short period of its life cycle (Urbaneja et al. 2013).

Several studies have shown that *Bacillus thuringiensis* Berliner subsp. *kurstaki* controls *T. absoluta* effectively in South America, as well as in the Mediterranean region (Niedmann Lolas & Meza-Basso 2006; González-Cabrera et al. 2011). Weekly applications of 90.4 MIU l^{-1} (million International Units per litre) were found to be sufficient for effective control of *T. absoluta* (González-Cabrera et al. 2011). Furthermore *B. thuringiensis* (*Bt*) is recommended as part of control strategies in IPM programs e.g. in Iran at low infestation levels of <10 moths/trap/week (Baniameri & Cheraghian 2012) or additionally to the release of *Trichogramma* in Brazil (Urbaneja et al. 2013).
Lo Bue et al. (2012) studied the efficacy of Azadirachtin to control *T. absoluta* concluding that Azadirachtin alone provides no satisfactory control, Azadirachtin mixed with *Bt* on the other hand showed promising results in open field conditions. Though, it has to be taken into consideration that the application of Azadirachtin is harmful not only to the pest, but also to natural enemies.
Another promising method in the biological control is the use of entomopathogenic fungi. *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin for example, reached mortalities of over 90% on *T. absoluta* larvae (Rodríguez et al. 2006).
Nematodes such as *Steinernema carpocapsae* Weiser, *Steinernema feltiae* Filipjev, and *Heterorhabditis bacteriophora* Poinar caused high larval mortality of up to 100% as well.

Furthermore nematodes are able to reach the larvae inside of the leaf galleries and therefore provide particularly efficient control of *T. absoluta* (Batalla-Carrera et al. 2010).

Pheromone-based control strategies

Pheromones are not only used for monitoring, but can also be part of control strategies.
In isolated greenhouses and at low population densities, mass trapping can be an effective control method (Urbaneja et al. 2013). Navarro Llopis et al. (2010) showed that mating disruption is a possible control strategy in greenhouses, provided that the greenhouses are properly isolated.

An approach to reduce the amount of insecticides needed for a successful control is the attract-and-kill strategy. Female-produced sex-pheromones are used to lure males into physical contact with an insecticide inadvertently killing the male. Since both sexes are able to mate several times and only males are attracted by the pheromone, a high proportion of the population has to be captured, to obtain an effective and throughout control (Witzgall et al. 2010).

Cultural control

The aim of most cultural practices is prophylactic control of the pest. Some wild-type tomatoes, as well as some commercial genotypes were found to hold resistance traits against *T. absoluta* (Urbaneja et al. 2013). To prevent plants from getting infested in the first place, seedlings have to be healthy and pest free. Fields should be kept free of weeds as they potentially serve as host plants for *T. absoluta*. Residues of infested crops have to be removed directly after the harvest and sealed in plastic bags exposed to direct sunlight. Tomato production should alternate with non-host crops in a suitable crop rotation (Urbaneja et al. 2013). Additionally, ploughing, adequate fertilization, and irrigation are recommended as cultivation measures against *T. absoluta* (EPPO 2005).

Integrated pest management strategies

IPM strategies against *T. absoluta* have already been developed in South America (EPPO 2005). These strategies combine the control measures mentioned above. They should include prophylactic measures and promote the conservation of natural enemies. Therefore aiming to reduce the use of curative pesticide treatments and emphasize the important role of biological control measures and resistance management strategies instead (Lietti et al. 2005; Desneux et al. 2010). One drawback of IPM is the labour intensity, comparatively making chemical based treatments by spray calendars far less complicated and more user friendly (Cunningham 1995). Nevertheless, developing suitable IPM strategies is all the more important, as an effective control of *T. absoluta* relying mainly on one control strategy will be difficult (Urbaneja et al. 2013).

3.3 Baculoviruses

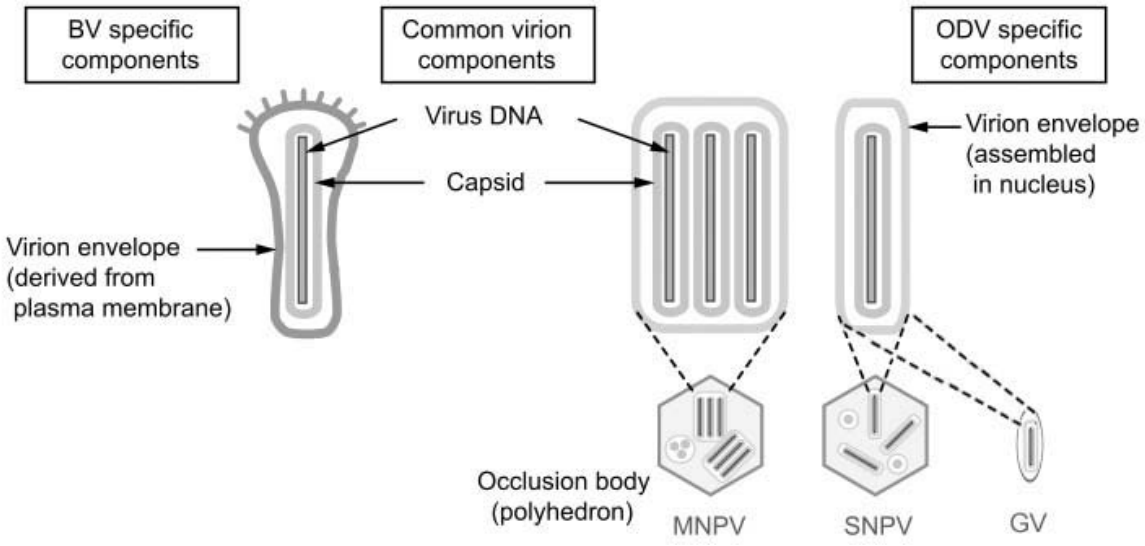
Baculoviridae are insect-pathogenic viruses and form one of the largest and most diverse groups among the virus families (Herniou et al. 2012; Ikeda et al. 2015). Phylogenetic analysis showed that the family of the Baculoviridae consists of 4 monophyletic groups. These groups can also be distinguished by morphological features and by the taxonomic classification of their insect host. Therefore baculoviruses are classified into the following four genera: *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus* (Herniou et al. 2012).
Granuloviruses are part of the genus *Betabaculoviruses*. They have been isolated from over 100 host species, all of them belonging to the lepidopteran insect order (Bonning 2005; Herniou et al. 2012; Ikeda et al. 2015). The other three genera comprise nucleopolyhedroviruses, specific to the insect orders Lepidoptera *(Alphabaculovirus)*, Hymenoptera (*Gammabaculovirus*), and Diptera (*Deltabaculovirus*), respectively (Herniou et al. 2012).
According to phylogenetic relationships, baculoviruses apparently evolved around 310 million years ago in coevolution with their holometabolous host insects (Okano et al. 2006; Thézé et al. 2011).
Baculoviruses are traditionally named according to their morphological characteristics and the host species from which they were isolated for the first time. Nevertheless, the name provides only limited information about the host species, since some viruses have a wider host range and infest more than one species. Likewise, some insect species are permissive for infection by different baculoviruses (Bonning 2005; Ikeda et al. 2015).

3.3.1 Characteristics of baculoviruses (and granuloviruses in particular)

Morphology

Baculoviruses are occluded in a protein matrix, also called occlusion body (OB). The shape of this envelope differs within the genera: *Alpha*-, *Gamma*- and *Deltabaculoviruses* have a polyhedral shaped protein envelope, containing multiple virions. Therefore they are called nucleopolyhedroviruses (NPVs). NPV occlusion bodies are 0.15-15 µm big (Herniou et al. 2012; Ikeda et al. 2015). *Betabaculoviruses* are surrounded by a smaller, ovicylindrical protein matrix, which contains only one (rarely more) virions. They are called granuloviruses (GVs) (Herniou et al. 2012; Ikeda et al. 2015). GV occlusion bodies, also called granules, have a size of 0.13x0.5 µm and a diameter of 0.2 – 0.4 µm (Herniou et al. 2012; Haase et al. 2015).
The virions within the OBs contain rod-shaped nucleocapsids (Herniou et al. 2012). Virions embedded in NPVs may consist of multiple nucleocapsids (in case of MNPVs) or single nucleocapsids (in case of SNPVs).
Nucleocapsids on their part are also enclosed within an envelope (Herniou et al. 2012). They have a tubular shape with a diameter of 30-60 nm, a length of 250-300 nm and contain the

genome, consisting of circular, supercoiled, double-stranded DNA (Bonning 2005; Herniou et al. 2012). Baculovirus genome size is between 80 to 180 kbp, *Betabaculoviruses* genome in particular has a length of 110-180 kbp (Herniou et al. 2012).



(modified from Ikeda et al. (2015))

Fig. 7: Schematic representation of BVs, ODVs, MNPVs, SNPVs and GVs.

During the baculovirus infection, two different phenotypes of virions occur: occlusion-derived virions (ODV) and budded virions (BV). Both occur at different stages in the infection-, propagation- and transmission cycle (Bonning 2005). Both phenotypes have the same nucleocapsid structure and carry identical genetic information, but differ in their envelope. ODV envelopes are assembled in the nucleus, whereas BV envelopes derive from the plasma membrane (Ikeda et al. 2015). BVs contain a single nucleocapsid, whereas ODVs consist of single or multiple nucleocapsids, enclosed by an envelope (Ikeda et al. 2015). ODVs are produced in the late phase of infection and play a key role in the primary infection of midgut cells and the horizontal transmission of the virus between host insects (Bonning 2005; Inceoglu et al. 2006; Ikeda et al. 2015). Virus propagation in the host cells, transmission within the host, and secondary cell infection takes place in form of BVs, which are produced during the early stage of infection (Bonning 2005; Inceoglu et al. 2006; Ikeda et al. 2015).

OBs protect the viral DNA against external factors as for example chemical and physical decay, extreme temperature and desiccation (Petrik et al. 2003; Herniou et al. 2012). To a certain extent, the OB also provides protection against inactivation by UV light. Though in general baculoviruses have rather short half-life times due to fast degradation (Ignoffo 1992; Kroschel et al. 1996; Herniou et al. 2012).

Infection cycle and dissemination within the host

Only the larval stage of insects is susceptible to baculovirus infection (Bonning 2005). Usually the larvae take up occlusion bodies of GVs and NPVs orally with contaminated food, though NPVs can also be transmitted vertically (Kukan 1999; Fuxa et al. 2002; Bonning 2005). Parent-to-offspring passage of virus is possible by transmission through infected ovaries (transovarial) or presumably also through infected males or through contaminated eggshells infecting the larvae throughout the hatching (Reed 1971; Khurad et al. 2004).
After ingestion the alkaline pH of the insect midgut (pH 9.5-11.5) causes the dissolution of the OB matrix and the release of ODVs (Bonning 2005; Ikeda et al. 2015). To facilitate the penetration of the peritrophic membrane, consisting of chitin, proteins, glycoproteins, and proteoglycans (Wang & Granados 2001), some OBs release a viral enhancing factor called enhancin (Popham et al. 2001). After successfully crossing the peritrophic membrane, ODVs bind to the microvilli of the epithelial cells and release the nucleocapsids into the midgut cells by fusing with the cellular membrane (Herniou et al. 2012; Ikeda et al. 2015). Nucleocapsids move through the cytoplasm to the nucleus and uncoat at the nuclear membrane to release the viral DNA (Bonning 2005). Viral gene expression, DNA replication and formation of new nucleocapsids takes place in the nucleus (Herniou et al. 2012; Ikeda et al. 2015).

Lepidopteran NPVs and almost all GVs initially infect the midgut, before spreading to other tissue (Bonning 2005). Usually the virus undergoes one replication cycle in the midgut epithelium cells before transmission to secondary tissues starts (Herniou et al. 2012).
Secondary infections are induced by BVs. Newly assembled nucleocapsids leave the midgut epithelium cells by budding from the basolateral plasma membrane of the infected cell as BVs (Herniou et al. 2012). From there the BVs start secondary infections of other susceptible tissues via the hemocoel (Herniou et al. 2012). Early in the secondary infection, nucleocapsids are dispersed from the infected cells as BVs as well (Ikeda et al. 2015). During the very late phase of infection large amounts of the major occlusion matrix protein polyhedrin (for *Alpha-* and *Gammabaculoviruses*) and granulin (for *Betabaculoviurses*) are produced (Bonning 2005; Haase et al. 2015). At this point nucleocapsids do not leave the infected cells as BVs and instead ODVs are formed, which are then enveloped to form OBs (Ikeda et al. 2015).
Once the baculovirus-infected host dies, virus encoded proteins cause liquefaction of the insect body and OBs with infectious ODVs are released into the environment (Herniou et al. 2012; Ikeda et al. 2015). One insect body, infected with granulovirus can release up to 10^10 OBs (Entwistle & Evans 1985). The amount of released OBs varies though depending on the species and the speed of kill of the host (Bonning 2005).

Several factors influence the response of the host to a baculovirus infection. The duration of the pathogenesis and the course of infection depend to a large extend on the host-virus combination, the larval instar, the virus dosis, as well as environmental conditions (Bonning 2005). A few days after an infection, the host insect becomes lethargic as a result of the virus dissemination

and replication. The cuticle turns glossy and swollen, and the caterpillar feeds lesser, until stopping food intake completely 2-4 days after infection. Infected caterpillars often climb to an exposed position on the plant and finally die 5-8 days after infection (Bonning 2005; Rosell et al. 2008). The onset of effect usually takes more time for GV (7-14 days) than for NPVs (Inceoglu et al. 2006). Facilitated by viral enzymes, the cuticle and internal tissues are degrading and the OBs are released from the lysed cells of the cadaver onto the plant tissue (Bonning 2005; Ikeda et al. 2015). Reed (1971) and Entwistle & Evans (1985) point out that predators and parasites of infected host insects also contribute in the dispersal of the virus.

Most baculoviruses encode proteins, which manipulate the host physiology and behaviour (Ikeda et al. 2015). The infection with a baculovirus for example lead to a suppressed host moulting and pupation by the inactivation of ecdysteroids (Bonning 2005). This furthermore can lead to increased feeding activity of the infected larvae, in comparison to healthy larvae, which cease feeding before the moult (Bonning 2005).
Usually baculoviruses are highly pathogenic and an infection results in the virus-induced death of the host (Entwistle & Evans 1985). However, sublethal infections are possible. Symptoms of sublethal infections may be reduced fecundity, a change in development time, lower pupal and adult weight as well as shorter adult longevity (Rothman & Myers 1996). Latent viruses can be transmitted vertically (Bonning 2005).

3.3.2 Use of baculoviruses in integrated pest management

Growing awareness of governments and the public on the negative side effects of chemical pesticides led to stricter regulations regarding insecticide use and an increasing interest in alternative pest control strategies in many countries. Implementation of IPM programs and organic production are gaining more support and users, as well as the industry become more interested in developing and applying biological insecticides (Moscardi 1999; Haase et al. 2015). Baculoviruses are known to be highly host specific and show a high insecticidal activity (Ikeda et al. 2015), contributing significantly in the natural control of insect pest populations (Inceoglu et al. 2006). Accordingly, the majority of commercial viral biopesticides are of the family Baculoviridae (Haase et al. 2015).

There are different strategies to control insects with baculoviruses (Moscardi 1999; Battu et al. 2002): Baculoviruses can be introduced and established in the ecosystem to supress the pest permanently. A less durable strategy is the seasonal colonization for long-term, but not permanent pest control. Another approach is the manipulation of the environment to conserve or reactivate a virus that is already present (Cunningham 1995; Battu et al. 2002). Furthermore the baculovirus can be used as microbial insecticide for a short-time control of the target pest, similar to chemical pesticides (Battu et al. 2002).

The establishment of viruses in an environment depends highly on the stability of the ecosystem and the host population as well as on the efficient transmission of the virus (Battu et al. 2002). Therefore long- or short-term establishment of baculoviruses is not suitable for all virus-host systems. In contrast most viruses can be used as microbial insecticides (Battu et al. 2002), as it was done in this study.

Worldwide, about 60 different baculoviruses are used as biological pest control agents (Beas-Catena et al. 2014). Nevertheless, baculoviruses still just have a small share of about 0.5% of the total pesticide market (Moscardi et al. 2011). Baculoviruses are widely used as biopesticides in Latin America. Some virus species control several pests in numerous crops. Virus, host, crop, and the country in which the virus is registered as a biocontrol product are listed in the table below (Table 2).

Table 2: List with examples of baculoviruses used as biological pesticides in Latin America.

VIRUS	HOST	CROPS	COUNTRY
Anticarsia gemmatalis MNPV	*Anticarsia gemmatalis*	Soybean	Brazil
Autographa californica MNPV + *Spodoptera albula* NPV	*Autographa californica* *Trichoplusia ni* *Pseudoplusia includens* *Heliothis virescens* *Spodoptera exigua* *Estigmene acrea* *Plutella xylostella*	Alfalfa, vegetable crops	Guatemala
Spodoptera sunia NPV	*Spodoptera* spp.	Vegetables	Guatemala
Cydia pomonella GV	*Cydia pomonella,* *Grapholita molesta*	Apple, pear, walnut, peach	Argentina, Chile, Uruguay
Erinnyis ello GV	*Erinnyis ello*	Cassava, rubber trees	Brazil, Colombia
Helicoverpa zea SNPV	*Heliothis* and *Helicoverpa* spp.	Maize, tomato, cotton, tobacco	Mexico, Brazil
Helicoverpa armigera NPV	*Heliothis* and *Helicoverpa* spp.	Tomato, sweet pepper, maize, soybean, tobacco, vegetable crops	Brazil
Phthorimaea operculella GV	*Phthorimaea operculella* *Tecia solanivora*	Potato	Colombia, Peru, Costa Rica
Phthorimaea operculella GV + *Bacillus thuringiensis*	*Phthorimaea operculella* *Tecia solanivora* *Symmetrischema tangolias*	Potato	Bolivia, Ecuador
Spodoptera exigua NPV	*Spodoptera exigua*	Tomato, chili, eggplant	Mexico
Spodoptera frugiperda MNPV	*Spodoptera frugiperda*	Maize, sorghum	Brazil

List adapted from Haase et al. (2015).

Similar to chemical insecticides, baculovirus-based pesticides are formulated for example by including additives (Entwistle & Evans 1985; Haase et al. 2015). The formulation aims to optimize storage ability, application, field stability, and uptake by the host (Haase et al. 2015). Since the virus has to be ingested, good spray coverage of the crop is essential to obtain efficient pest control (Cunningham 1995). For that reason the virus may be mixed with surfactants, adherents, thickeners, as well as UV protectants and phagostimulants (Haase et al. 2015).

Since the 1980s genetic modification of baculoviruses for pest control purposes has been studied (Haase et al. 2015). Several studies suggest that it might improve the host range and increase the speed of kill (Bonning 2005; Inceoglu et al. 2006). Promising results were achieved with the transfer of genes for insect-specific toxins, metabolic enzymes, and hormones, which interfere with the physiology, development, and behaviour of the host (Inceoglu et al. 2006; Ikeda et al. 2015).
Genetically modified pest control agents are subjected to strict legal regulations (Haase et al. 2015). Even though studies so far have not given any indication that recombinant baculoviruses pose a higher risk than wild type baculoviruses, the lack of public acceptance might be a problem for the practical implementation (Moscardi et al. 2011).

3.3.3 Granuloviruses against *T. absoluta*

*Phop*GV

The granulovirus of the potato tuber moth *Phthorimaea operculella* (Zeller) (*Phop*GV) was discovered in *Phthorimaea operculella* populations and was considered a potential control agent against the potato tuber moth (Briese 1981; Alcázar et al. 1991). Later it was introduced as microbial pesticide through an initiative of the CIP, since it is highly efficient against *P. operculella* in the field and in the storage. *Phop*GV is commercially available as a viral pesticide against the potato tuber moth in potato cultivars and is nowadays widely used in Latin America (Battu et al. 2002; Haase et al. 2015).
*Phop*GV was found to infect other species within the family of the Gelechiidae as well, such as *Tecia solanivora*. Particularly *Phop*GV has shown a high virulence against *T. absoluta* (Angeles Pava & Alcázar Sedaño 1996; Zeddam et al. 2003; Mascarin et al. 2010).

*Tuab*GV

Until today, there is only little information available about *T. absoluta* Granulovirus (*Tuab*GV) and its efficacy against the tomato leafminer *T. absoluta*.

In 2014, Gómez-Valderrama et al. reported the first detection of *Tuab*GV in field samples of *T. absoluta* in Colombia, suggesting that *Tuab*GV might provide better control of the tomato leafminer than other GV isolates, since it is better adapted to the host and the tomato plant.

3.3.4 Advantages and disadvantages of GV as plant protection agents

Advantages

Baculoviruses show a high insecticidal activity (Ikeda et al. 2015). At the same time, they are considered to be safe and environmentally sound pesticides (Haase et al. 2015). Due to their narrow host range, they have no or little effect on beneficials and other non-target organisms, including vertebrates and plants (Haase et al. 2015). They are arthropod-specific and have not been found in other animals, plants, fungi or bacteria (Ikeda et al. 2015). The host range usually is limited to one species or only a few insect species within the same family (Ikeda et al. 2015; Haase et al. 2015). GVs and hymenopteran NPVs seem to have an even narrower host range than lepidopteran NPVs (Bonning 2005).

As already described above (see Control methods in chapter 3.2.1), resistance against common chemical pesticides is an increasing problem, which can be defused by developing more sustainable control strategies, including biological control mechanisms.

In contrast to other biological control approaches, such as predators and parasitoids, viruses can be mass produced, formulated, packaged, and stored similar to chemical control agents (Beas-Catena et al. 2014).

For all these reasons baculoviruses can be considered effective insecticides, with the advantages of biological control agents i.e. not being harmful for neither the environment nor the user and consumer. At the same time baculoviruses provide easier handling in comparison to the usage of natural enemies.

Disadvantages

One major drawback of baculoviruses is the relatively slow speed of kill in contrast to chemical insecticides (Battu et al. 2002). Depending on the virus, the host, the larval instar, and the temperature, it can take 3 to 20 days for the baculoviruses to kill the host (Cunningham 1995), whereas for example pyrethroids kill the pest within hours after application (Inceoglu et al. 2006).

Due to the suppression of moulting in infected larvae, sometimes feeding activity of infected larvae even increases before death occurs (Bonning 2005). On the other hand Entwistle & Evans (1985) quote several sources confirming mortality time lags in baculovirus treatments which however did not result in higher yield losses, compared to chemical treatments.

Persistence in the field is a crucial factor for the success of a control agent. Baculoviruses are susceptible to degradation by UV-light and therefore have a short field stability. Viral activity can be lost completely in less than 24 hours, yet on average half-lifes mostly between 2-5 days have been observed (Moscardi et al. 2011). Therefore frequent applications are required to maintain the insecticidal activity in the field (Inceoglu et al. 2006).
Even though virus storage and transport is less critical than for living organisms, viral insecticides have a rather limited shelf-life in comparison to chemicals (Cunningham 1995).
In the end production cost, which is closely linked to the total costs of the insecticide application, is a crucial factor for the successful implementation of a control strategy. In comparison to chemical insecticides, the production costs for baculoviruses are relatively higher (Inceoglu et al. 2006).
The narrow host range mentioned above, can also be a disadvantage, if a field is affected by different pest species. In this case a coapplication of several baculovirus species for throughout control is required (Moscardi 1999; Inceoglu et al. 2006). An improvement could be achieved by developing multispecies-baculovirus formulations, which would allow the simultaneous biological control of several pests (Haase et al. 2015).

Limitations

Successful use of baculoviruses as biopesticides depends on several factors, including the selection of the most effective virus isolate, as well as the application timing (Moscardi et al. 2011). Especially when controlling boring insects such as *T. absoluta*, accurate timing is crucial, since the virus solution only covers the plant surface and a lethal dosis has to be taken up before the insect enters the plant tissue (Moscardi 1999). Reed (1971) states, though that granuloviruses can enter the plant tissue through stomata and infect mining larvae. Still, frequent monitoring is required to ensure that the larvae are exposed to the virus right after their emergence (Moscardi et al. 2011). This makes the use of viral insecticides more labour intensive than the application of chemicals by a spray calendar (Cunningham 1995). Experiencing the lower efficacy and the slower speed of kill of viral insecticides, farmers may decide to prefer rapid killing chemical insecticides. Therefore educational programs are necessary to inform farmers about the long-term advantages of biological control methods (Cunningham 1995; Moscardi et al. 2011).
To oppose the effect of a slow speed of kill, baculoviruses can be combined with other biological control methods or with conventional insecticides (Cunningham 1995; Zechendorf 1995; Battu et al. 2002). In the later case interactions between the virus and those insecticides have to be studied before it can be recommended for pest control (Battu et al. 2002).

The future perspectives for baculovirus based pesticides are promising. Biopesticides have gained popularity in high value agricultural products and demand is likely to increase for commercially available biopesticides in small and large horticultural farms in the future as well (Haase et al. 2015).

3.4 Aim of the study

The aim of this study was to assess the potential use of different isolates of *P. operculella* Granulovirus (*Phop*GV) and *T. absoluta* Granulovirus (*Tuab*GV) in integrated pest management in tomato cropping against *T. absoluta*.
Therefore, the biological activity of five different isolates of *Phop*GV (*Phop*GV La Molina, *PhopGV* Huaraz, *PhopGV* Huancayo, *Phop*GV Cuzco, *Phop*GV Chile) and two isolates of *Tuab*GV (*Tuab*GV La Molina, *Tuab*GV St. Rita (Arequipa)) was tested to detect the isolates with the highest pathogenicity. Those isolates, which showed the highest efficacy in the first laboratory bioassay, were tested further on tomato plants under controlled conditions. To gain further information about their possible use in integrated pest management, the activity against the four larval instars of *T. absoluta,* as well as the virus stability were tested.

T. absoluta is a threat to successful tomato production in all countries, where the pest has spread so far. Farmers are threatened by severe damages and yield losses in case of *T. absoluta* infestation. Since tomato is an important vegetable crop worldwide, the development of sustainable IPM strategies for the control of *T. absoluta* benefits large-scale tomato production and secures income and nutrition of small- and medium-scale tomato growers.

Seeing that in the past an efficient control of *T. absoluta* has proven to be difficult, only relying on chemical insecticides (Zlof & Suffert 2012), the availability of an integrated management strategy is of particular importance. Hence, this study aims to contribute to the development of alternative control strategies for *T. absoluta* in tomato cropping in the future.

Expected results
It was expected to find variation between the different isolates in their biological activity against *T. absoluta*. The high biological activity of the best isolates against *T. absoluta* had to be confirmed in the following tests on tomato plants.
Furthermore eventual differences in the susceptibility of the four larval instars of *T. absoluta* against the granulovirus were tested. Several studies indicate that virus-induced mortality decreases with increasing larval age (Sait et al. 1994; Sporleder et al. 2007). Thus, first instar larvae were expected to be more susceptible than later instars.
The third trial was carried out to reveal the speed of the virus degradation after application. Since granuloviruses are known to have a rather low field stability (usually a half-life of 2-5 days according to Moscardi et al. (2011)), a fast degradation of the granulovirus was expected.

4 MATERIALS AND METHODS

***T. absoluta* population**

All experiments were carried out with insects from a *T. absoluta* population, reared in the laboratory of the CIP's experimental station in La Molina, Lima. This population had its origin from a field population collected from the same station. Since the CIP conducts several studies about *T. absoluta*, a mass rearing of this pest is maintained.

The mass rearing was done in a greenhouse in wooden cages at an average temperature of 25°C ± 0.31 and 74% HR. 7-8 week old tomato plants were placed in cages and infested with *T. absoluta* adults. Two weeks later the leaves were cut and put on a grating on top of a plastic container filled with a shallow layer of sand. *T. absoluta* nymphs left the tomato leaves to pupate in the sand. One week later the pupae were harvested by sieving the sand. The pupae could be kept at 10°C for up to 15 days before using them for experiments or for further mass rearing. The pupae were put individually in small plastic boxes until the adults emerged. Typically an emergence rate of ca. 70% was obtained and the adults had a female to male-ratio of 2:1.

For further rearing, adults were put in plastic containers with 1-2 tomato branches for mating and oviposition. The eggs laid onto the tomato leaves could be used for experiments or for infestation of new plants for rearing purposes.

Plant material

Plant material was obtained from tomato plants of the variety "Rio Grande", which were grown in CIP greenhouses. Once a week tomato seeds were sown in potting soil (Growing mix SOGEMIX SM-2, Premier Tech Horticulture, Canada). After 3-4 weeks, seedlings were transplanted (three seedlings per 4" pot). After 7-8 weeks plants were used for the mass rearing or individual leaves or branches were cut as food for the larvae in the laboratory experiments.

Virus isolates

The isolates *Phop*GV La Molina, *Phop*GV Chile, *Phop*GV Cuzco, *Phop*GV Huancayo, *Phop*GV Huaraz, *Tuab*GV La Molina, and *Tuab*GV St. Rita (Arequipa) were tested within the context of this thesis. The virus isolates were field isolates from different South American origins. The place of origin is noted in the isolates name.

The virus samples were stored in the CIP laboratory at -34°C and propagated yearly by infecting potato tuber moth larvae. To obtain the desired concentration of virus occlusion bodies for the bioassays, the stock solution was diluted in distilled water and the OBs/ml were counted with a Neubauer chamber under a phase-contrast microscope. Then the necessary dilution to obtain the desired concentration was calculated.

Pre-tests

Before starting the bioassays, pre-tests were carried out to test the methodology and to verify that treated larvae showed symptoms of the virus infection.

The pre-test confirmed that the GV isolates cause infection of *T. absoluta*. Infected larvae showed symptoms of a virus infection in the form of a whitish colouring and a flabby consistency of the larvae tissue.

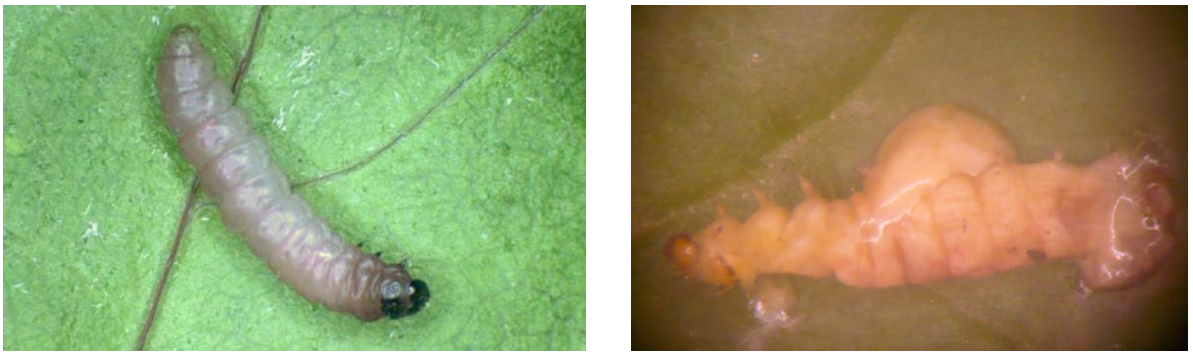


Fig. 8: *Phop*GV infected *T. absoluta* larvae.

4.1 Egg dip assay

The egg dip assay was carried out in two replications. In the first experiment the six isolates *Phop*GV La Molina, *Phop*GV Chile, *Phop*GV Cuzco, *Phop*GV Huancayo, *Phop*GV Huaraz, and *Tuab*GV La Molina were tested. *Phop*GV La Molina and *Phop*GV Chile were tested in 3 repetitions and *Phop*GV Cuzco, *Phop*GV Huancayo, *Phop*GV Huaraz, and *Tuab*GV La Molina were tested in 4 repetitions. All isolates were tested in five concentrations (2.50E+05 OBs/ml, 5.00E+06 OBs/ml, 7.50E+07 OBs/ml, 3.00E+08 OBs/ml, and 6.15E+08 OBs/ml). The control was treated with distilled water.

The concentrations were prepared as dilutions of the stock solutions. The stock solution was diluted eleven times at the ratio of 1:1 with distilled water. The number of OBs per ml in the highest dilution was counted with a Neubauer chamber under a phase-contrast microscope. Afterwards, the concentration of OBs per ml in the stock solution was calculated as well as the required dilutions for the desired concentrations for the bioassay. Each concentration was prepared corresponding to the calculations.

For the treatment, about 80 eggs of *T. absoluta* for each dilution were transferred with a fine brush from tomato leaves onto filter paper (1/4 of Ø 90mm filter). The eggs were treated with 500 µl of the respective virus dilution. The filter was air-dried for approximately 30 minutes. Then the filter paper was set on a paper towel for a few seconds to remove the remaining moisture. The filter was then placed in a new sterile petri dish, which was sealed with parafilm afterwards. The eggs were incubated for 4-5 days at 25°C until the larvae hatched.

2-3 tomato leaves (with 5 leaflets each) were placed in a petri dish (Ø 15 cm) with a paper towel on the ground. A piece of cotton, wrapped in aluminium foil around the stem provided necessary moisture (Fig. 9). Depending on the availability approximately 50 larvae per petri dish were carefully transferred with a fine brush onto the leaves and the dishes were sealed with parafilm. Petri dishes were kept at 25°C and 12:12 hours L:D regime in the climatic chamber.
The number of surviving and dead larvae as well as the number of pupae (and adults, if present) was assessed under the binocular 5, 10, 15, and 20 days after the transfer of the larvae. Dead larvae were removed from the petri dish. Larvae were assumed to be dead if they did not react to careful tactile stimuli with fine tweezers.
When the tomato leaves wilted or did not provide enough food anymore, they were replaced with fresh leaves.

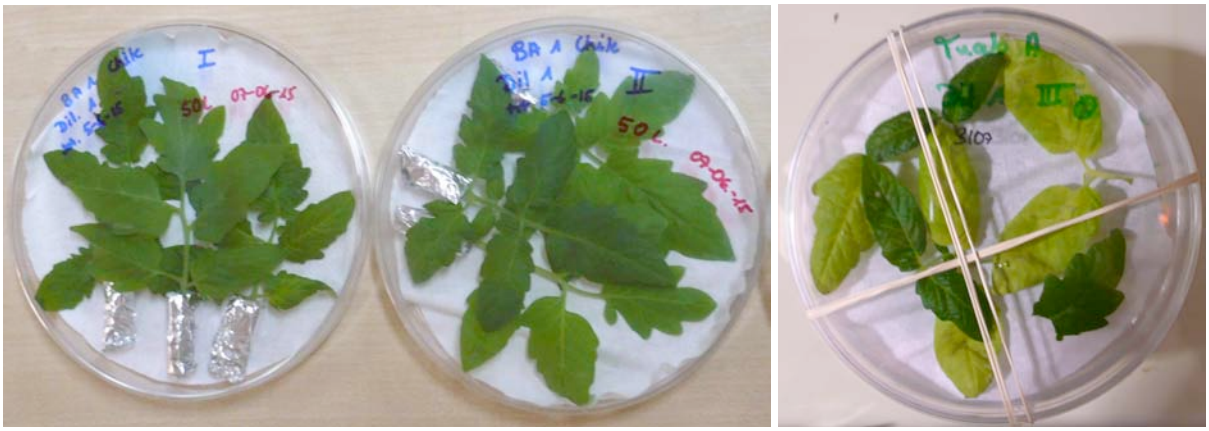


Fig. 9: Petri dishes in the first (left) and the second (right) egg dip assay.

For the second egg dip bioassay the methodology was slightly adjusted.
The implementation was similar to the first experiment, but only five larvae were transferred to each petri dish. To simplify the handling, five individual tomato leaflets were placed in the dishes, instead of one tomato leaf with five leaflets. One larva was put on each leaflet. Petri dishes were closed first with elastics to provide exchange of air and prevent too high humidity inside the petri dishes (Fig. 9). Again, the leaves were replaced when wilted or eaten up.

Petri dishes were kept at 25°C and a L:D regime of 9:15 hours in the laboratory. Later (after 9 days) the dishes were closed with parafilm to prevent the larvae from escaping the petri dishes.
Additionally to the six isolates used in the first replication, another isolate of *Tuab*GV from St. Rita (Arequipa) was tested. All variants were tested in 12 repetitions.

4.2 Susceptibility test

Subsequent to the egg dip bioassays, the susceptibility of the four larval instars of *T. absoluta* against the best GV isolates was tested. The two isolates *Phop*GV Huancayo and *Tuab*GV La Molina were applied with the concentration 6.15E+08 OBs/ml.
Tomato plants were repotted into 3.5" plastic pots containing potting soil (Growing mix SOGEMIX SM-2, Premier Tech Horticulture, Canada). Plants with a height of approximately 15 cm were infested each with five L1 larvae of *T. absoluta*. All variants were prepared in four repetitions. Each repetition consisted of two plants. The infested plants were placed separately in plastic containers (4 litres). These were topped with nylon gauze to provide exchange of air and to hinder the larvae from escaping as well as preventing infestation with other insects (Fig. 10).

Several studies agree on the finding that each larval instar takes approximately three days before changing into the next larval instar, when kept at approximately 25°C (Table 3).

Table 3: Duration (days) of the larval instars in three life table studies of *T. absoluta*.

	Larval instar				**Conditions**	
	L1	**L2**	**L3**	**L4**	Temperature (°C)	Humidity (% RH)
Vargas (1970)	3.6	2.9	3.0	3.7	25.1	74.7
Erdogan & Babaroglu (2014)	2.49±0.09	2.32±0.07	2.52±0.07	3.79±0.19	25-26	60-70
Çekin & Yasar (2015)	3.11-3.56	3.00-3.13	2.53-3.24	2.71-3.13	24-26	60-70

Plants for the susceptibility tests of L1 larvae were treated with the virus dilutions and distilled water (Control) directly before infesting them with L1 larvae. For testing the susceptibility of L2 larvae, plants infested with L1 larvae of *T. absoluta* were kept in the plastic containers for 3 days before treating them with the same amount of virus dilution. For testing L3 and L4 larvae the plants infested with L1 larvae were kept in the laboratory for 6 and 9 days before treatment.
The plants were treated with 3.8 ml of virus dilution (6.15E+08 OBs/ml) each, using a hand-held aerosol (Fig. 11). To provide better wetting of the leaf surface, 0.2% Triton-X100 was added.

Treated plants were put back into the plastic containers and were kept in the laboratory at 25°C and 9:15 hours L:D regime. When the plants were beginning to wilt or the larvae had eaten most of the leaves, the plants were cut and placed in the containers together with a new, untreated tomato plant.

Fig. 10: Experimental setup for the susceptibility test.

Fig. 11: Hand-held aerosol.

Surviving larvae, dead larvae, and number of pupae and adults were assessed on all plants 15 days after infestation with the L1 larvae. For the assessment, the leaves were cut and examined under the binocular microscope. Additionally, the plastic containers and pots were checked for larvae or pupae. Furthermore the containers with the pot inside were kept for another seven days, so that pupae, which were hidden in the soil, had time to hatch. Adults, which were found after these seven days, were considered as pupae at the time of evaluation.

4.3 Stability test

Another test was planned to assess the stability of the virus over time. Due to cold temperatures the *T. absoluta* mass rearing was slowed down, leading to a delayed implementation of the experiment. Therefore the experiment was completed after the scheduled experimentation period and the results were kindly made accessible to be presented in this study.

The stability of the most promising virus isolates was tested by releasing *T. absoluta* adults on virus-treated tomato plants in different time intervals after virus application. The same isolates used in the second experiment regarding the larval susceptibility (*Phop*GV Huancayo and *Tuab*GV La Molina) were selected for the stability testing.

Tomato plants were treated in 5 repetitions with the hand-held spraying device with 3.8 ml of virus dilution (concentration: 6.15E+08 OBs/ml) each. Treated plants were placed in plastic containers (4 litres) with nylon gauze on top (similar setup like in the susceptibility test).

After 0, 2, 5, and 7 days, three pairs of *T. absoluta* adults were released for 24 hours onto each plant. The plants remained in the plastic containers and were kept in the laboratory at 25°C and a L:D regime of 9:15 hours afterwards. If the plants wilted or did not provide sufficient food for

the larvae anymore, the plant material was renewed during the experimentation period by cutting the treated and infested plants and placing them into the plastic container together with a new, untreated plant.
After 22 days the population density of *T. absoluta* in the containers was assessed. Additionally the infection rate and pupation rate was assessed for each treatment.

4.4 Statistics

The efficacy of the virus isolates was calculated according to the formula of Schneider-Orelli (1947):

$$efficacy\ (\%) = \frac{mortality\ (\%)\ treatment - mortality\ (\%)\ control}{100 - mortality\ (\%)\ control}$$

Statistical analysis was carried through with the program jmp® (SAS Institute Inc.). Data was analysed and tested by performing a GLM. Normal distribution was tested with the Shapiro-Wilk test. Normally distributed data was analysed by a one-way ANOVA and a Welch-test (in case of unequal variances). Means were subsequently compared with a Student t-test. Not normally distributed data was analysed by performing a Kruskal-Wallis test, followed by a pairwise Wilcoxon signed rank test.
The significance was set at a probability level of $P \leq 0.05$.

A pobit-analysis (Finney 1971) was conducted for the results of the egg dip bioassay (second experiment, 15 days after transfer of infected larvae onto tomato leaves) to determine the LC_{50}, LC_{95}, and LC_{99}.

5 RESULTS

5.1 Egg dip assay

First experiment

GLM showed that all three factors isolate, concentration, and date of assessment had significant influence on the efficacy of the virus (Table 4).

Table 4: GLM of the efficacy in the first egg dip assay.

Source	DF	L-R Chi-squared	Likelihood > X^2
Isolate	5	81.762454	<.0001
OB/ml	1	27.929991	<.0001
Days after transfer of larvae	3	32.707711	<.0001

N=412, P≤0.05.

Table 5 displays the comparison of mean efficacies between the virus isolates (columns) and the virus concentrations (rows) five days after the transfer of the infected larvae onto the tomato leaves. The mean efficacies of the isolates differed extremely between the isolates and the concentrations, but due to high variance, the differences were often not significant. Especially *Tuab*GV La Molina had conspicuously high standard deviations (up to 102.95) in all concentrations.

All *Phop*GV isolates, except for *Phop*GV La Molina, differed not significantly from one another in the lower concentrations (2.50E+05 up to 3.00E+08 OBs/ml). At low concentrations, *Tuab*GV La Molina was the least efficient isolate.

In general, efficacies varied extremely within the isolates and concentrations, so that no clear ranking of the isolates regarding their efficacy became apparent. The differences between the mean efficacies of the isolates were most clearly in the highest concentration. At 6.15E+08 OBs/ml *Phop*GV La Molina (82.96%) was as effective as *Phop*GV Huancayo (40.35%), but significantly more effective than *Phop*GV Huaraz (31.45%), *Phop*GV Cuzco (-2.67%), *Phop*GV Chile (-10.17%), and *Tuab*GV La Molina (-63.64%).

Control mortalities higher than the mortality in the respective treatment resulted in negative efficacies of some isolates.

The comparison of the efficacy at different virus concentrations showed an irregular development for the most isolates. An increase of efficacy with rising virus concentrations could not be detected.

Table 5: Mean efficacies (%) and standard deviation of the means (SD) of the six virus isolates 5 days after the transfer of the infected larvae (dpt) onto tomato leaves.

Isolate	Virus concentration (OBs/ml)							
	2.50E+05	**5.00E+06**	**7.50E+07**	**3.00E+08**	**6.15E+08**	**F**	**DF**	**P**
***Phop*GV Chile**	-10.64 Ca (4.52)	-8.34 Ca (6.15)	11.69 Bab (7.41)	27.81 Aa (4.80)	-10.17 Cc (5.25)	26.9351	4, 10	<.0001
***Phop*GV Cuzco**	-23.46 Ba (21.62)	42.10 Aa (18.66)	60.44 Aa (13.31)	3.30 Ba (23.83)	-2.67 Bbc (29.25)	8.3195	4, 14	0.0012
***Phop*GV Huancayo**	18.84 BCa (18.33)	50.00 Aa (14.47)	30.44 ABCab (10.04)	8.70 Ca (6.90)	40.35 ABab (19.71)	5.0129	4, 15	0.0091
***Phop*GV Huaraz**	10.63 Ba (11.67)	48.17 Aa (12.18)	4.88 Bab (8.21)	3.77 Ba (9.19)	31.45 Abc (21.11)	8.5277	4, 15	0.0009
***Phop*GV La Molina**	20.37 BCa (29,30)	-14.06 Dab (9.24)	-0.25 CDb (13.19)	39.78 Ba (3.96)	82.96 Aa (9.48)	15.8020	4, 9	0.0004
***Tuab*GV La Molina**	-130.30 Cb (73.40)	-84.07 BCb (102.95)	25.13 Aab (74.61)	24.24 ABa (38.81)	-63.64 ABCd (54.66)	3.6208	4, 15	0.0295
F	5.066	19.369	7.7623	10.7111	10.1			
DF	5, 7	5, 7	5, 7	5, 6	5, 16			
P	0.0286	0.0004	0.0106	0.0047	0.0002			

Means within a column followed by the same lower case letter are not significantly different. Means within a row followed by the same capital letter are not significantly different. ($P \leq 0.05$).

Comparison of mean efficacies 10 days after the transfer of the infected larvae is presented in Table 6. After 10 days, the differences between the isolates were more clearly than after 5 days. *Phop*GV Huancayo was one of the most efficient isolates in all concentrations with mean efficacies of 45.36-100.00%. *Phop*GV Huaraz showed rather good activity of up to 94.57%, as well. *Phop*GV Cuzco, *Phop*GV Chile, *Phop*GV La Molina, and *Tuab*GV La Molina had more irregular mean efficacies, showing higher activity in some concentrations, but lower in others.

Same as in the earlier assessment, rising concentrations did not necessarily result in increasing virus efficacy. For *Phop*GV La Molina and *Tuab*GV La Molina (and to a lesser extend for *Phop*GV Huancayo and *Phop*GV Huaraz) a tendency towards rising efficacy with increasing concentrations could be observed.

The values of *Phop*GV Cuzco, *Phop*GV Huancayo, and *Phop*GV Huaraz were unexpectedly high for 5.00E+06 OBs/ml.

Table 6: Mean efficacies (%) and standard deviation of the means (SD) of the six virus isolates 10 days after the transfer of the infected larvae (dpt) onto tomato leaves.

Isolate	**Virus concentration (OBs/ml)**							
	2.50E+05	**5.00E+06**	**7.50E+07**	**3.00E+08**	**6.15E+08**	**F**	**DF**	**P**
***Phop*GV Chile**	32.08 ab (10.55)	20.57 b (26.07)	-3.60 (11.96)	25.18 a (9.97)	9.06 c (26.15)	1.7338	4, 10	0.2188
***Phop*GV Cuzco**	-33.87 Dc (26.24)	81.41 Aa (11.72)	48.96 AB (22.64)	-7.94 CDa (19.19)	14.47 BCc (25.08)	17.4831	4, 14	<.0001
***Phop*GV Huancayo**	46.39 Ca (10.10)	100.00 Aa (0.00)	45.36 C (14.03)	56.70 BCa (15.25)	68.81 Bab (9.61)	16.2591	4, 15	<.0001
***Phop*GV Huaraz**	13.84 Bab (33.32)	94.57 Aa (5.47)	41.30 B (43.26)	30.44 Ba (18.45)	43.48 Bbc (29.06)	4.3559	4, 15	0.0155
***Phop*GV La Molina**	-5.37 BCbc (26.06)	-1.31 BCb (46.42)	-16.26 C (20.27)	42.59 ABa (4.78)	91.72 Aa (7.69)	8.0350	4, 9	0.0048
***Tuab*GV La Molina**	-125.00 Cd (50.00)	-82.03 BCc (61.57)	33.33 A (47.14)	0.00 ABa (105.41)	66.67 Aab (27.22)	11.0166	4, 7	0.0035
F	11.4337	18.0371	2.4390	5.2603	6.8416			
DF	5, 7	5, 16	5, 15	5, 7	5, 16			
P	0.0030	<.0001	0.0829	0.0262	0.0014			

Means within a column followed by the same lower case letter are not significantly different. Means within a row followed by the same capital letter are not significantly different. (P≤0.05).

15 days after the transfer of larvae, the mean efficacies differed significantly for the concentrations 5.00E+06 OBs/ml, 3.00E+08 OBs/ml, and 6.15E+08 OBs/ml (Table 7). *Phop*GV Huancayo was one of the best isolates in all those concentrations with 92.78-100.00% mean efficacy.

For 5.00E+06 OBs/ml, *Phop*GV Cuzco, *Phop*GV Huancayo, and *Phop*GV Huaraz showed unexpectedly high activities again and were significantly more efficient than *Tuab*GV La Molina, *Phop*GV La Molina, and *Phop*GV Chile. For 3.00E+08 OBs/ml however, *Tuab*GV La Molina together with *Phop*GV Huancayo was significantly more efficient than the other isolates tested. For 6.15E+08 OBs/ml *Phop*GV Chile showed a significantly lower efficacy than all other isolates.

Table 7: Mean efficacies (%) and standard deviation of the means (SD) of the six virus isolates 15 days after the transfer of the infected larvae (dpt) onto tomato leaves.

Isolate	**Virus concentration (OBs/ml)**							
	2.50E+05	**5.00E+06**	**7.50E+07**	**3.00E+08**	**6.15E+08**	**F**	**DF**	**P**
***Phop*GV Chile**	43.39 (44.09)	-45.98 b (36.12)	-25.02 (32.27)	19.56 b (23.65)	-28.11 b (67.08)	2.2199	4, 10	0.1397
***Phop*GV Cuzco**	18.78 (33.45)	100.00 a (0.00)	47.94 (23.59)	32.78 b (40.91)	49.59 a (53.73)	2.9115	4, 14	0.0604
***Phop*GV Huancayo**	82.47 B (7.04)	100.00 Aa (0.00)	82.47 B (11.84)	92.78 Aa (3.95)	96.91 Aa (3.95)	5.9808	4, 15	0.0044
***Phop*GV Huaraz**	30.83 CD (20.30)	94.77 Aa (3.49)	0.58 D (45.35)	49.42 BCb (32.41)	77.33 ABa (18.35)	7.1858	4, 15	0.0019
***Phop*GV La Molina**	-15.29 C (10.87)	-2.25 Cb (27.28)	7.91 BC (13.72)	33.64 Bb (13.41)	93.01 Aa (6.94)	20.7523	4, 9	0.0001
***Tuab*GV La Molina**	-33.33 B (108.87)	0.00 ABb (66.67)	-33.33 B (108.87)	100.00 Aa (0.00)	100.00 Aa (0.00)	3.3158	4, 15	0.0391
F	2.3609	13.4922	2.3998	6.4153	6.5874			
DF	5, 16	5, 16	5, 15	5, 15	5, 16			
P	0.0872	<.0001	0.0866	0.0022	0.0017			

Means within a column followed by the same lower case letter are not significantly different. Means within a row followed by the same capital letter are not significantly different. (P≤0.05).

Table 8 shows the mean efficacies 20 days after the transfer of larvae. Significant differences between the isolates were only found in the lower concentrations. At 5.00E+06 OBs/ml, *Phop*GV Huancayo and *Phop*GV Cuzco were significantly more efficient than *Phop*GV Chile. However, for the concentration of 7.50E+07 OBs/ml, *Phop*GV Chile and *Phop*GV Cuzco had significantly higher efficacies than *Phop*GV Huaraz.

*Tuab*GV La Molina was not assessed after 20 days, because no surviving larvae were left after 15 days.

Table 8: Mean efficacies (%) and standard deviation of the means (SD) of the five virus isolates 20 days after the transfer of the infected larvae (dpt) onto tomato leaves.

Isolate	Virus concentration (OBs/ml)							
	2.50E+05	**5.00E+06**	**7.50E+07**	**3.00E+08**	**6.15E+08**	**F**	**DF**	**P**
***Phop*GV Chile**	33.65 a (57.46)	-65.87 c (50.67)	100.00 ab (0.00)	39.18 (70.23)	37.34 (49.86)	2.9761	4, 10	0.0737
***Phop*GV Cuzco**	50.00 a (27.36)	42.86 ab (61.72)	100.00 a (0.00)	64.29 (27.36)	78.57 (42.86)	1.1877	4, 14	0.3587
***Phop*GV Huancayo**	42.86 BCa (15.55)	80.95 ABa (21.99)	33.33 Cbc (50.40)	95.24 A (9.52)	95.24 A (9.52)	8.3961	4, 7	0.0076
***Phop*GV Huaraz**	20.56 BCab (28.25)	81.90 Aa (13.02)	-26.72 Cc (54.98)	32.76 AB (48.15)	76.72 AB (27.21)	5.6337	4, 15	0.0057
***Phop*GV La Molina**	-25.76 Cb (11.98)	5.07 Bb (14.30)	21.58 Bbc (8.70)	78.33 A (18.39)	94.29 A (4.94)	51.8479	4, 9	<.0001
F	10.1536	8.7021	6.0185	2.2775	1.7342			
DF	4, 6	4, 13	4, 11	4, 5	4, 13			
P	0.0075	0.0012	0.0081	0.2050	0.2024			

Means within a column followed by the same lower case letter are not significantly different. Means within a row followed by the same capital letter are not significantly different. (P≤0.05).

Considered all three dates of assessment, *Phop*GV Huancayo was the isolate that showed the highest activity most frequently. *Phop*GV Huaraz and *Phop*GV Cuzco displayed rather good activity, too. *Tuab*GV La Molina was the isolate, which was most frequently least effective. *Phop*GV Chile and *Phop*GV La Molina generally showed a rather low efficacy as well. Nevertheless, no general ranking regarding the efficacy of the virus isolates could be established, since the values fluctuated rather irregularly.
Variation of the efficacy was quite high in most variants. Especially the values of *Tuab*GV La Molina varied extremely (see standard deviations in Table 5-8).

High mortality in the untreated control resulted quite frequently in negative mean efficacies of the virus treatments.

Influence of the date of assessment was significant as well (see GLM, Table 4). Though a comparison of means is not presented here. Efficacy of the virus isolates increased with progressing time. Most isolates (*Phop*GV Cuzco, *Phop*GV Huancayo, *Phop*GV Huaraz, and *Tuab*GV La Molina) showed a more definite increase of efficacy with progressing time at higher virus concentrations.

Second experiment

The GLM analysis showed that all three factors isolate, concentration, and date of assessment had significant influence on the efficacy of the virus (Table 9).

Table 9: GLM of the efficacy in the second egg dip assay.

Source	DF	L-R Chi-squared	Likelihood > X^2
Isolate	6	146.44851	<.0001
OBs/ml	4	85.94229	<.0001
Days after transfer of larvae	2	164.51171	<.0001

N=1188, P≤0.05.

Five days after larval transfer, there were significant differences of mean efficacy in all concentrations (Table 10). *Tuab*GV La Molina (with mean efficacies of 4.44-21.52%) and *Phop*GV Huancayo (with mean efficacies of 3.15-27.44%) were two of the most efficient isolates in all concentrations. *Tuab*GV St. Rita (Arequipa) was quite efficient, as well. Contrary to this, *Phop*GV Chile was one of the least efficient isolates in all concentrations, with values between -3.45% and 0.86%. Highest mean efficacy after five days was 30.41% for 6.15E+08 OBs/ml of the isolate *Tuab*GV St. Rita (Arequipa).

Five days after the transfer of larvae, significant differences between the concentrations could only be observed in *Tuab*GV St. Rita (Arequipa), where the mean efficacy in the lowest concentration differed significantly from the mean efficacy in the highest concentration.

Table 10: Mean efficacies (%) and standard deviation of the means (SD) of the seven virus isolates 5 days after the transfer of the infected larvae (dpt) onto tomato leaves.

Isolate	Virus concentration (OBs/ml)					X^2	DF	P
	2.50E+05	5.00E+06	7.50E+07	3.00E+08	6.15E+08			
***Phop*GV Chile**	-3.45 (0.00)	-3.45 (0.00)	-3.45 (0.00)	-1.72 (5.97)	0.86 (14.93)	3.0523	4	0.5491
***Phop*GV Cuzco**	8.33 (18.01)	0.00 (0.00)	1.82 (6.03)	6.25 (15.54)	0.00 (0.00)	6.1092	4	0.1911
***Phop*GV Huancayo**	3.15 (26.92)	13.19 (22.69)	6.01 (17.62)	27.44 (35.98)	15.48 (32.73)	5.7287	4	0.2203
***Phop*GV Huaraz**	-0.45 (11.40)	-2.25 (10.68)	-2.21 (19.56)	-3.24 (10.33)	8.11 (16.78)	6.7931	4	0.1472
***Phop*GV La Molina**	0.00 (5.87)	1.70 (7.92)	1.41 (7.29)	0.00 (5.87)	10.17 (16.13)	6.7654	4	0.1488
***Tuab*GV La Molina**	4.44 (10.76)	15.00 (17.32)	15.28 (18.12)	17.73 (16.94)	21.52 (12.57)	8.5324	4	0.0739
***Tuab*GV St. Rita (Arequipa)**	-1.72 (5.97)	7.62 (17.82)	8.97 (32.64)	5.17 (16.41)	30.41 (38.85)	9.8538	4	0.0430
X^2	40.7165	29.1386	37.5010	32.0445	18.5730			
DF	6	6	6	6	6			
P	<.0001	<.0001	<.0001	<.0001	0.0049			

Analysis of variance performed by the Kruskal-Wallis test (P≤0.05). For comparison of means by pairwise Wilcoxon test see Table A2 – Table A13 in the annex.

Mean efficacies 10 days after the transfer of larvae are presented in Table 11. The isolates showed significant differences in the all virus concentrations. Similar to the first assessment (5 dpt), *Tuab*GV La Molina and *Phop*GV Huancayo were two of the most efficient isolates in all concentrations with mean values of 7.78-57.79% and 7.42-44.95%, respectively. *Tuab*GV St. Rita (Arequipa) had equally high mean efficacies of 6.58-48.54%. Least efficient isolates were *Phop*GV Cuzco (-2.13-14.89%), *Phop*GV La Molina (-2.66-15.93%), and *Phop*GV Huaraz (-2.75-20.77%).

For the *Phop*GV isolates from Chile and La Molina, as well as for *Tuab*GV La Molina, an increasing efficacy with rising concentrations could be observed.

Table 11: Mean efficacies (%) and standard deviation of the means (SD) of the seven virus isolates 10 days after the transfer of the infected larvae (dpt) onto tomato leaves.

Isolate	Virus concentration (OBs/ml)							
	2.50E+05	**5.00E+06**	**7.50E+07**	**3.00E+08**	**6.15E+08**	**X^2**	**DF**	**P**
***Phop*GV Chile**	-4.96 (0.00)	-2.77 (7.57)	13.27 (20.92)	25.95 (33.13)	17.78 (25.16)	18.1740	4	0.0011
***Phop*GV Cuzco**	6.38 (18.39)	0.71 (9.83)	1.59 (8.26)	14.89 (36.11)	-2.13 (0.00)	2.9205	4	0.5712
***Phop*GV Huancayo**	7.42 (31.90)	41.12 (40.40)	24.92 (36.04)	44.95 (41.40)	38.78 (41.28)	6.5087	4	0.1642
***Phop*GV Huaraz**	13.76 (22.76)	2.14 (19.30)	9.76 (25.13)	-2.75 (15.47)	20.77 (30.50)	5.9723	4	0.2012
***Phop*GV La Molina**	-2.21 (9.37)	1.77 (11.87)	-2.66 (8.27)	0.44 (16.50)	15.93 (22.28)	10.7816	4	0.0291
***Tuab*GV La Molina**	7.78 (16.29)	33.76 (43.64)	36.74 (42.38)	33.96 (30.02)	57.79 (39.12)	10.3606	4	0.0348
***Tuab*GV St. Rita (Arequipa)**	6.58 (18.42)	22.35 (32.21)	9.79 (26.77)	22.15 (39.52)	48.54 (41.44)	8.9636	4	0.0620
X^2	19.5894	21.6542	18.9584	26.5761	14.1157			
DF	6	6	6	6	6			
P	0.0033	0.0014	0.0042	0.0002	0.0284			

Analysis of variance performed by the Kruskal-Wallis test ($P \leq 0.05$). For comparison of means by pairwise Wilcoxon test see Table A14 – Table A25 in the annex.

15 days after the larval transfer, the isolates showed significantly different mean efficacies in all concentrations (Table 12). *Phop*GV Huancayo and *Tuab*GV La Molina again were two of the best isolates in all concentrations, with mean efficacies of 12.32-76.31% and 24.17-72.68%, respectively. *Tuab*GV St. Rita (Arequipa) was one of the best isolates in all concentrations, but at a relatively high concentration of 3.00E+08 OBs/ml efficacy was low. Same as in the

assessment after 10 days, *Phop*GV Cuzco was one of the least efficient isolates, with -11.77-23.50% mean efficacy.
An increase in efficacy with rising concentrations could be observed for the *Phop*GV isolates from Chile, Huancayo, and La Molina, as well as for both *Tuab*GV isolates.

Table 12: Mean efficacies (%) and standard deviation of the means (SD) of the seven virus isolates 15 days after the transfer of the infected larvae (dpt) onto tomato leaves.

Isolate	Virus concentration (OBs/ml)							
	2.50E+05	**5.00E+06**	**7.50E+07**	**3.00E+08**	**6.15E+08**	**X^2**	**DF**	**P**
***Phop*GV Chile**	1.58 (25.21)	18.60 (33.41)	33.70 (30.54)	51.11 (41.16)	66.93 (25.03)	22.0543	4	0.0002
***Phop*GV Cuzco**	0.66 (23.18)	0.82 (24.62)	-11.77 (14.10)	23.50 (48.63)	-9.29 (26.23)	6.6911	4	0.1531
***Phop*GV Huancayo**	12.32 (45.01)	53.12 (43.36)	62.53 (41.28)	76.31 (29.78)	59.02 (45.24)	10.7866	4	0.0291
***Phop*GV Huaraz**	38.24 (31.35)	15.57 (27.89)	29.86 (41.23)	22.16 (43.07)	34.47 (26.23)	3.6665	4	0.4530
***Phop*GV La Molina**	-11.28 (19.72)	-3.93 (15.85)	32.48 (26.57)	53.01 (43.86)	36.52 (29.48)	27.2357	4	<.0001
***Tuab*GV La Molina**	24.17 (34.11)	27.87 (52.10)	53.61 (41.48)	40.20 (38.00)	72.68 (34.70)	9.0982	4	0.0587
***Tuab*GV St. Rita (Arequipa)**	14.24 (37.13)	71.70 (44.75)	77.36 (30.55)	25.58 (39.87)	69.81 (37.17)	17.1854	4	0.0018
X^2	23.4043	22.7611	30.0406	13.4720	28.9123			
DF	6	6	6	6	6			
P	0.0007	0.0009	<.0001	0.0361	<.0001			

Analysis of variance performed by the Kruskal-Wallis test ($P \leq 0.05$). For comparison of means by pairwise Wilcoxon test see Table A26 – Table A37 in the annex.

Considering all dates of assessment *Phop*GV Huancayo, followed by *Tuab*GV La Molina and *Tuab*GV St. Rita (Arequipa) were the most efficient isolates. *Phop*GV Cuzco, *Phop*GV La Molina, *Phop*GV Chile, and *Phop*GV Huaraz most often ranked among the least efficient isolates.
Increasing concentration resulted not inevitably in a significant increase of efficacy. However, even when no significant differences could be detected, the mean values mostly had an increasing tendency with increasing concentration, especially in the later assessments (15 dpt).
Negative efficacies occurred, but the values were not as extremely low as in the first experiment.

The date of assessment influenced the mean efficacy of the isolates as well (see GLM, Table 9). However, the comparison of means is not presented here. The course of the mean efficacy over

the time is presented exemplary for the isolates *Phop*GV Huancayo and *Tuab*GV La Molina in the highest virus concentration (6.15E+08 OBs/ml) in Fig. 12.

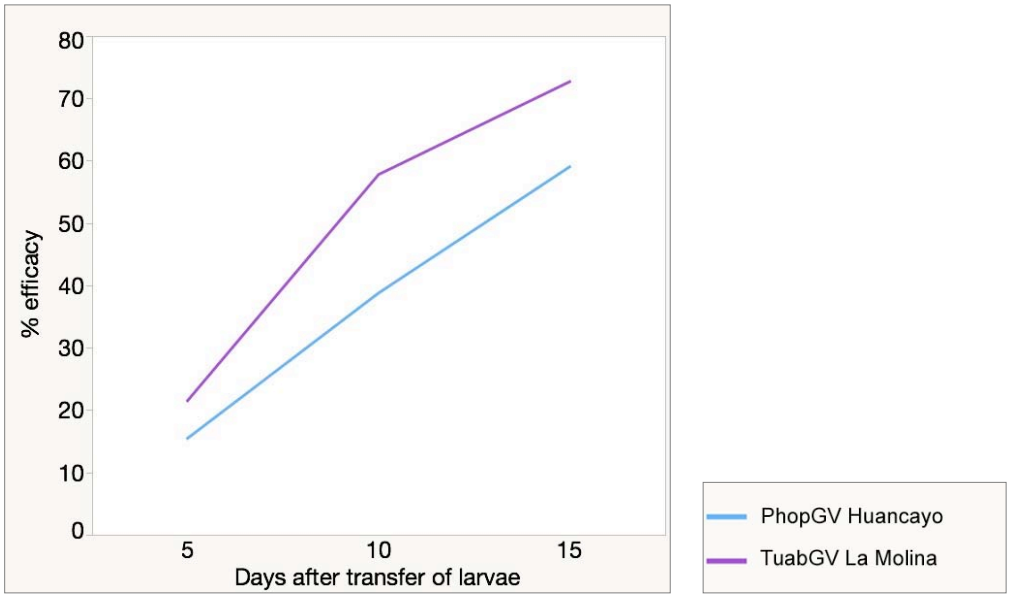


Fig. 12: Course of the mean efficacy of the isolates *Phop*GV Huancayo and *Tuab*GV La Molina at the concentration 6.15E+08 OBs/ml.

Probit analysis

For the data of the assessment 15 days after the transfer of larvae, a probit analysis (Finney 1971) was conducted to determine the LC_{50}, LC_{95} and LC_{99} (Table 13). LC_{50} (or LC_{95} and LC_{99}) is the virus concentration required to kill 50% (or 95% and 99%, respectively) of the pest population.

Probit analysis resulted in LC_{50} values within a range of 4.27E+08 OBs/ml (*Phop*GV Huancayo) to 2.05E+09 OBs/ml (*Phop*GV Huaraz). LC_{95} values ranged between 1.95E+09 OBs/ml (*Phop*GV Cuzco) and 9.56E+09 OBs/ml (*Phop*GV Huaraz), whereas LC_{99} values varied between 2.23E+09 OBs/ml (*Phop*GV Cuzco) and 1.27E+10 OBs/ml (*Phop*GV Huaraz).

Table 13: Results of probit analysis: LC_{50}, LC_{95}, and LC_{99} in OBs/ml on basis of the assessment 15 days after the transfer of the infected larvae onto tomato leaves (second egg dip bioassay).

Isolate	Equation[1]	LC $_{50}$	LC $_{95}$	LC $_{99}$	r^2 corrected
*Phop*GV Chile	y = 4.4612 + 9.996e-10x	5.39E+08	2.18E+09	2.87E+09	0.126396
*Phop*GV Cuzco	y = 4.1213 + 1.440e-9x	6.10E+08	1.75E+09	2.23E+09	0.178144
*Phop*GV Huancayo	y = 4.7213 + 6.533e-10x	4.27E+08	2.94E+09	3.99E+09	0.019415
*Phop*GV Huaraz	y = 4.5531 + 2.182e-10x	2.05E+09	9.56E+09	1.27E+10	-0.01655
*Phop*GV La Molina	y = 4.0699 + 1.307e-9x	7.12E+08	1.97E+09	2.49E+09	0.150928
*Tuab*GV La Molina	y = 4.3664 + 4.843e-10x	1.31E+09	4.69E+09	6.12E+09	-0.01145
*Tuab*GV St. Rita (Arequipa)	y = 4.5395 + 8.645e-10x	5.33E+08	2.43E+09	3.23E+09	0.112791

[1]y=probit efficacy; x=OBs/ml.

Linear regression of the probit efficacies by concentration resulted in straight lines with a rather low slope (Fig. 13).

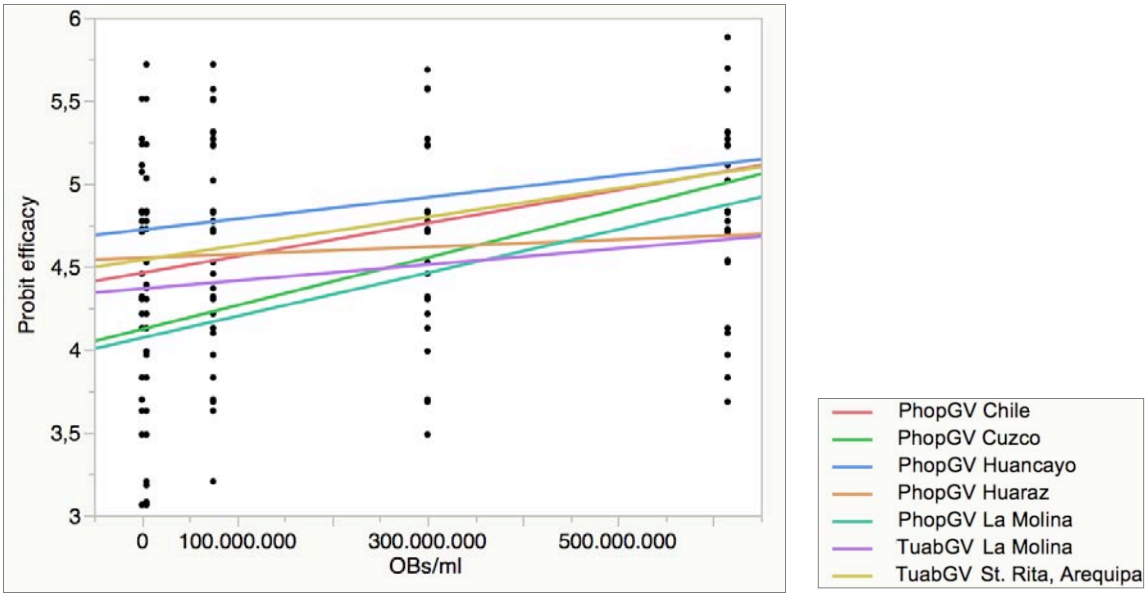


Fig. 13: Linear regression of the probit efficacy by concentration for all seven isolates 15 days after the transfer of the infected larvae onto tomato leaves.

According to these calculations, *Phop*GV Huaraz required the highest concentrations for efficient control. LCs of *Tuab*GV La Molina ranged in higher concentrations, as well. In contrast, *Phop*GV Cuzco and *Phop*GV La Molina required lower concentrations.
LC_{50} values of all isolates ranged close to the highest concentration tested in this bioassay (6.15E+08 OBs/ml).

5.2 Susceptibility of the four larval instars

The GLM showed that the influence of the isolate was significant, whereas the influence of the larval instar was not (Table 14).

Table 14: GLM of the efficacy in the susceptibility test of the different larval instars.

Source	DF	L-R Chi-squared	Likelihood > X^2
Isolate	1	7.8693628	0.0050
Larval instar treated	3	7.2544571	0.0642

N=32, P≤0.05.

Table 15 shows the comparison of means for the isolates *Phop*GV Huancayo and *Tuab*GV La Molina and the four larval instars treated.
The Kruskal-Wallis test revealed significant differences in the mean efficacies of the two isolates only for the first larval instar. L1 larvae showed a high susceptibility against *Phop*GV Huancayo (80.77% mean efficacy), but a significantly lower susceptibility against *Tuab*GV (-7.69% mean efficacy).
The efficacies in the second to fourth instar differed not significantly from another. The efficacy in the second instar was a bit higher than 20% for both isolates and increased in the third instar to around 50% for both isolates. In the fourth instar, the efficacy was again higher for *Phop*GV Huancayo (32.46%) than for *Tuab*GV La Molina (3.51%), but the difference was not significant.

According to the pairwise Wilcoxon test, significant differences in the susceptibility of the larval instars could be observed. For *Phop*GV Huancayo, the first instar was the most susceptible one, followed by the third than the second and the fourth instars. For *Tuab*GV La Molina, the third instar was more susceptible than the first, the second, and the fourth instars. Thus, the third instar larvae (and the first instar in case of *Phop*GV Huancayo) seemed to be more susceptible than the other instars, whereas the second and the fourth instar larvae were less susceptible.

Table 15: Mean efficacies (%) and standard deviation of the means (SD) of the isolates *Phop*GV Huancayo and *Tuab*GV La Molina against the four larval instars.

Treatment	**Larval instar treated**						
	1st instar	**2nd instar**	**3rd instar**	**4th instar**	**X^2**	**DF**	**P**
***Phop*GV Huancayo**	80.77 A (19.36)	24.24 B (18.18)	48.72 AB (30.19)	32.46 B (12.32)	8.6128	3	0.0349
***Tuab*GV La Molina**	-7.69 B (30.77)	21.21 B (12.12)	56.41 A (9.82)	3.51 B (30.63)	9.8584	3	0.0198
X^2	5.6709	0.0921	0.0222	1.7943			
DF	1	1	1	1			
P	0.0172	0.7615	0.8817	0.1804			

Analysis of variance by Kruskal-Wallis test. Comparison of means by pairwise Wilcoxon test. Means within a row followed by the same capital letter are not significantly different. ($P \leq 0.05$).

5.3 Virus stability

Efficacy

The GLM showed that neither the isolate, nor the days after treatment had a significant influence on the efficacy of the virus (Table 16). Therefore the mean efficacies are displayed in Table 17 without comparison of means.

Table 16: GLM of the efficacy in the stability test.

Source	DF	L-R Chi-squared	Likelihood > X^2
Isolate	1	0.1374549	0.7108
Days after treatment	3	5.8284705	0.1203

N=40, P≤0.05.

The mean efficacy ranged from 52.84%-71.03% and 41.03%-92.50% for *Phop*GV Huancayo and *Tuab*GV La Molina, respectively. With progressing time, no significant change in the virus activity could be observed.

Table 17: Mean efficacies (%) and standard deviation of the means (SD) of the two virus isolates from 0 to 7 days after treatment (dpt).

Treatment	Days after treatment			
	0 dpt	2 dpt	5 dpt	7 dpt
***Phop*GV Huancayo**	52.84 (25.52)	71.03 (25.79)	70.70 (13.05)	66.59 (33.26)
***Tuab*GV La Molina**	68.67 (16.54)	69.75 (17.95)	41.03 (26.48)	92.50 (16.77)

Infection rate

The GLM performed for the infection of the treated larvae revealed a significant influence of the isolate, whereas the days after treatment did not influence the infection significantly (Table 18). This GLM included the data of the treatments and the untreated control. If only the virus treatments were considered, the influence of the isolate would not be significant (data not shown).

Table 18: GLM of the infection in the stability test.

Source	DF	L-R Chi-squared	Likelihood > X^2
Isolate	2	259.22325	<.0001
Days after treatment	3	6.6562603	0.0837

N=60, P≤0.05.

No infection was observed in the untreated control. In contrast, both virus treatments resulted in high infection levels, with mean values between 90.64% - 100.00% for *Phop*GV Huancayo and 95.39% - 100.00% for *Tuab*GV La Molina. At all days both isolates were significantly more infectious than the untreated control. At the same time, the infection levels of the virus treated variants were not significantly different from one another. Thus, virus treatment caused high infection levels, while the infection rate was not affected by time.

Table 19: Mean infection (%) and standard deviation of the means (SD) of untreated (Control) and treated (*Phop*GV Huancayo, *Tuab*GV La Molina) larvae from 0 to 7 days after treatment (dpt).

Treatment	Days after treatment				X^2	DF	P
	0 dpt	2 dpt	5 dpt	7 dpt			
Control	0.00 b (0.00)	0.00 b (0.00)	0.00 b (0.00)	0.00 b (0.00)	0.0000	3	1.0000
***Phop*GV Huancayo**	90.64 a (10.01)	100.00 a (0.00)	92.67 a (10.11)	100.00 a (0.00)	6.4847	3	0.0903
***Tuab*GV La Molina**	95.39 a (10.32)	97.14 a (6.39)	98.18 a (4.07)	100.00 a (0.00)	1.1385	3	0.7678
X^2	10.7722	12.6667	11.1306	14.0000			
DF	2	2	2	2			
P	0.0046	0.0018	0.0038	0.0009			

Analysis of variance by Kruskal-Wallis test. Comparison of means by pairwise Wilcoxon test. Means within a column followed by the same letter are not significantly different (P≤0.05).

Pupation rate

The GLM performed for the pupation (Table 20), revealed a significant influence of the isolate, but no significant influence of the time. This GLM, like the GLM of the infection, also included the data of the virus treatments as well as the data of the untreated control. If only the virus treatments were considered, the influence of the isolate would not be significant (data not shown).

Table 20: GLM of the pupation in the stability test.

Source	DF	L-R Chi-squared	Likelihood > X^2
Isolate	2	234.58193	<.0001
Days after treatment	3	7.483487	0.0580

N=60, P≤0.05.

Pupation rate was significantly higher in the untreated control (with mean values of 92.64-98.07%) than in the virus treated variants, regardless of the time past virus application. *Phop*GV Huancayo and *Tuab*GV La Molina both showed low pupation rates between 0.00% and 7.69% that did not differ significantly between the isolates. Thus, virus treatment lowered the pupation rate significantly, while the isolate had no significant influence. Furthermore, time gone by between the treatment and the actual exposure of the moth towards the virus, had no influence on the pupation rate either.

Table 21: Mean pupation (%) and standard deviation of the means (SD) of untreated (Control) and treated (*Phop*GV Huancayo, *Tuab*GV La Molina) larvae from 0 to 7 days after treatment (dpt).

Treatment	**Days after treatment**				X^2	**DF**	**P**
	0 dpt	**2 dpt**	**5 dpt**	**7 dpt**			
Control	98.07 a (2.78)	97.01 a (4.18)	95.76 a (7.22)	92.64 a (11.87)	0.3640	3	0.9476
***Phop*GV Huancayo**	7.69 b (10.88)	0.00 b (0.00)	7.33 b (10.11)	0.00 b (0.00)	4.6769	3	0.1970
***Tuab*GV La Molina**	4.62 b (10.32)	0.00 b (0.00)	1.82 b (4.07)	0.00 b (0.00)	2.1158	3	0.5487
X^2	10.6108	13.4271	10.7744	13.4271			
DF	2	2	2	2			
P	0.0050	0.0012	0.0046	0.0012			

Analysis of variance by Kruskal-Wallis test. Comparison of means by pairwise Wilcoxon test. Means within a column followed by the same letter are not significantly different ($P \leq 0.05$).

6 DISCUSSION

6.1 Methodological aspects

The examination of the biological activity of the different virus isolates was carried through an egg dip bioassay. The eggs were treated with the diluted virus solution, so that the larvae took up the occlusion bodies during the hatching process by consuming parts of the chorion. Only successfully emerged larvae were transferred onto the tomato leaves in order to be ensured that all larvae used in the experiment had taken up occlusion bodies.

In advance of the actual bioassays, two forms of the egg dip method were tested. Transferring the eggs onto filter paper in order to treat them with the virus-dilution afterwards was found to be more practical than treating the eggs directly on tomato leaves by dipping the leaves in the virus solution. The virus-dilution appeared to spread more homogeneous on the filter. Furthermore the transfer of the infected larvae onto the experimental site was easier, as the larvae had no chance to start mining in the leaf before they were transferred.

Another approach was to infect L1 larvae by letting them feed on droplets of virus-dilution mixed with tomato extract. One could not be certain afterwards, which larvae had eaten from the treated droplets and were therefore infected. Besides some larvae drowned in the droplets while feeding on them.

In the chosen experimental methodology larvae only took up the virus during the process of emerging, since the tomato leaves they were transferred onto afterwards were untreated. As the virus was only applied on the eggs instead of onto the complete leaf surface, as it would be done in practical field application, the amount of virus particles taken up by the larvae is lower than it would be under field conditions. Nevertheless, aside from the above mentioned observations gained from the pretests, Entwistle & Evans (1985) stressed the advantages of the egg dip method, namely: high precision and low handling requirements. Furthermore, the egg dip method was already proved suitable for biological testing of *Phop*GV activity against *P. operculella* (Sporleder et al. 2005).

Egg dip assay

In the first experiment of the egg dip assay, around 50 larvae were placed in one petri dish. Tomato leaves were consumed quickly and the leftovers had dried or had started to rotten quickly, often before they could be replaced in time. Under these conditions finding all larvae for an accurate evaluation was difficult. Besides that, the high density of larvae contributed to an unfavourable microclimate inside the petri dishes, especially high moisture levels. High density, shortage in food, and inconvenient microclimate probably stressed the larvae and may have raised the natural mortality rate. This is why the number of individuals was reduced drastically to 5 larvae per petri dish for the second replication. Furthermore the assessments were stopped after 15 days, since virus-induced death should have occurred 14 days after the infection at the latest

(Inceoglu et al. 2006). Therefore the additional assessment after 20 days did not generate added value. Hence, the first experiment could be classified as test run, whereas the second experiment was considered to yield more reliable results.

Test of the susceptibility of the larval instars & Test of the virus stability

The susceptibility of the larval instars and the stability of the virus treatment, were examined on treated tomato plants. This way, the experimental set up was more similar to practical field applications. The treated plants were put in plastic containers to prevent the larvae (and adults) from escaping. Both experiments were conducted in the laboratory under controlled conditions adding to a high level of accuracy of the experiments.

For the treatment of the four larval instars, the time of application after the larval hatch was determined based on data from reviewed literature as well as personal experience during the pretests. Thus, the application was scheduled without checking if the larvae indeed had reached the desired instar. Larval development may not be absolutely synchronous, so that at the time of the virus application, some larvae may not have been in the instar, which should have been examined at that particular stage in the experiment.
L1 larvae were transferred onto freshly treated tomato plants and took up the virus when they started to feed on the leaf tissue. Since the L2, L3, and L4 larvae had the opportunity to enter the leaf tissue before the virus dilution was applied, they were in comparison possibly exposed to a smaller amount of virus. Therefore the experimental setup took into account not only the physiological susceptibility, but also possible differences in the susceptibility due to distinct larval behaviour.

Several factors influence the persistence of a virus, but UV radiation is considered one of the main factors regarding the decrease of biological activity of baculoviruses (Entwistle & Evans 1985). Since the stability experiment was conducted under artificial light, this factor was disregarded. Therefore, the results from the stability test are probably not reproducible under field conditions. To obtain information regarding UV-stability of the virus, the third experiment should be repeated outdoors under the influence of solar radiation.

The virus dilutions were applied with a simple hand-held aerosol. This spraying device was not optimal for these experiments, because the amount of OBs applied per plant could not be measured out as exactly as it would have been possible e.g. with an automated spraying device.

Experimental conditions

All three tests were carried out under controlled conditions. A temperature of 25°C was maintained, as it had been done in several previous studies with *T. absoluta* (Pereyra & Sánchez 2006; Visintin da Silva Galdino et al. 2011; Caparros Megido et al. 2013; Çekin & Yasar 2015). During the course of the study it became apparent that a slightly lower temperature may have been better for conducting the bioassays, because the larvae may have shown less locomotory activity at lower temperatures. Cuthbertson et al. (2013) stated an optimum temperature of 19-23°C for development of *T. absoluta*.

During the assessments, the number of dead and surviving larvae, as well as the number of pupae and adults (if present) were assessed to determine the efficacy of the virus isolates. In some cases of the egg dip bioassays and the test of the susceptibility of the larval instars, not all larvae, which were originally transferred, could be found at the time of the assessment. As mentioned above, the tomato leaves started to dry or rot quickly, especially when many larvae were feeding on the tissue simultaneously. Finding the dead larvae, especially those that had died in an early instar, was difficult.
Parafilm sealing the petri dishes mostly prevented the larvae from escaping during the experiments. Therefore it seems more probable that lost larvae were mostly dead, but not found and consequently lost larvae were considered dead at the time of assessment. Nevertheless, it cannot be ruled out that some larvae managed to escape the petri dishes alive and were mistakenly considered dead during the assessments. Since these potentially false estimations of the mortality numbers occurs in the untreated control likewise, it should not influence the estimation of the efficacy.

In some variants efficacies were not only low, but negative. This was most likely not due to an enhancing effect of the virus treatment, but caused by high control mortalities.
Possible reasons may be sensitive reactions to the unfavourable conditions in the petri dishes mentioned above (lack of space and food, high moisture), due to the high density of larvae. Healthy larvae in the untreated control were possibly fitter and more active than those in the treated variants, so that maybe more individuals managed to escape in the control.
Mortality due to handling may also contribute to high control mortalities. The larvae were transferred onto the leaves with a fine brush. Even with extremely careful handling, it cannot be ruled out that some larvae were damaged.
Another possible explanation for a high natural mortality rate is cannibalism, which already has been observed in various insect species. A high population density and to a lesser extend also lack of food are considered main factors for inducing cannibalism (Fox 1975).

In all three experiments a higher number of repetitions would have aided in achieving a higher statistical accuracy. The high variance of the data made the interpretation challenging. With a higher number of repetitions, results may become clearer and statistically more reliable.

6.2 Promising isolates

As expected, all three factors (virus isolate, virus concentration, and the date of assessment) had a significant influence on the virus efficacy.
The results of the first experiment were difficult to interpret, because none of the isolates or the virus concentrations were clearly notable as the most effective. Mean efficacies varied extremely within the different isolates and concentrations.

Previous studies reported that virus isolates from different geographical regions, show differences in their biological activity against the host insect (Zeddam et al. 2013; Sporleder 2003). Accordingly, the egg dip assay revealed differences in the activity of the various South American isolates tested in this study as well.
Considering both experiments and all dates of assessment, *Phop*GV Huancayo was one of the most efficient isolates among all tested concentrations. Additionally *Tuab*GV La Molina and *Tuab*GV St. Rita (Arequipa) showed higher efficacies against *T. absoluta* larvae than the other isolates tested. However, in the second experiment of the egg dip assay, highest efficacy on day five was 30.41% (*Tuab*GV St. Rita, (Arequipa)), 57.79% on day ten (*Tuab*GV La Molina), and 77.36% on day 15 (*Tuab*GV St. Rita (Arequipa)). Hence, none of the tested isolates showed real high efficacy. Even in the highest concentration tested (6.15E+08 OBs/ml), the efficacy would not be sufficient for the practical use as an insecticide. Further testing in higher concentrations is therefore essential.
In comparison to most *Phop*GV isolates, both *Tuab*GV isolates revealed higher activities against *T. absoluta*, which might suggest that *Tuab*GVs are particularly well adapted to their host. This assumption has to be confirmed by further examination of other *Tuab*GV isolates, once additional *Tuab*GV isolates are available. On the other hand, the high efficacy of *Phop*GV Huancayo confirmed previous findings about a potential high pathogenicity of *Phop*GV against *T. absoluta* (Angeles Pava & Alcázar Sedaño 1996; Zeddam et al. 2003; Mascarin et al. 2010).
In contrast, *Phop*GV Chile and *Phop*GV Cuzco showed rather low efficacies, especially during the second experiment. Yet ranking these isolates was more difficult due to quite similar biological activity, whereas isolates with higher efficacy were easier to identify.

Since the methodology was adjusted for the second experiment, results obtained under the improved conditions were considered more reliable.
Being the best isolates in the egg dip assay, *Phop*GV Huancayo and *Tuab*GV La Molina were considered most promising and were therefore chosen for further examination in the following tests.

6.3 Influence of time and virus concentration on the efficacy of the GVs

In general, granuloviruses have a rather slow effect on their hosts. It takes 7-14 days until death occurs (Inceoglu et al. 2006). This may be the reason why the results were unclear 5 days after the transfer of the larvae. With progressing time trends regarding the efficacy of the isolates became more distinct.
In some variants of the first experiment, control mortality increased towards the end of the experiment, causing a decrease of efficacy from the third (15 days after the transfer of larvae) to the fourth assessment (20 days after the transfer of larvae). Mortalities in the untreated control probably resulted from unfavourable conditions in the petri dishes. Since granulovirus usually show an effect within 7-14 days the second experiment was stopped after the third assessment.

Higher virus concentrations were expected to cause higher efficacy against *T. absoluta*, as it already had been observed in previous studies with *Phop*GV (e.g. Sporleder et al. 2007; Mascarin et al. 2010). A similar trend was observed in this experiment, even though the effect did not come out as clearly for all isolates. In the first experiment unexpectedly high mean efficacies were observed at 5.00E+06 OBs/ml for the *Phop*GV isolates from Cuzco, Huancayo, and Huaraz. Those particular isolates had to be repeated separately in a second run due to a calculation mistake. In theory the second attempt at the virus preparation was carried out exactly the same as for the other isolates and concentrations. However the results give reason to the suspicion that some mistakes were made during calculation, preparation or application of the virus dilution during the second run, especially since this observation did not recur in the second experiment.

Lethal concentrations

The probit analysis resulted in LC_{50} values of 4.27E+08 - 2.05E+09 OBs/ml, ranging close to the highest concentration tested in the egg dip bioassay. LC_{95} and LC_{99} values were calculated at 1.95E+09 - 9.56E+09 OBs/ml and 2.23E+09 - 1.27E+10 OBs/ml, respectively. Thus concluding that higher concentrations than those tested in this bioassay, would be required to kill 95% and 99% of the pest population, respectively.
A study from Brazil reported much lower LC values: Mascarin et al. (2010) found a LC_{50} of *Phop*GV against *T. absoluta* of 1.05E+04 OBs/ml and a LC_{90} of 2.66E+06 OBs/ml. A comparison of these results though can only be made with reservations, as the experimental methodology was not identical. Furthermore, the LC_{50} and LC_{95} values calculated in this study, are much closer to each other than those described by Mascarin et al. Since in our study, the calculated LC_{95} and LC_{99} were outside the range of the concentrations tested in the egg dip assay, the results should be considered with caution. In contrast, the calculated lethal concentrations from the Brazilian study ranged within the tested concentration range and therefore might be more reliable. Hence, further tests with a wider range of concentrations,

especially including concentrations higher than 6.15E+08 OBs/ml have to be conducted to confirm the theoretical LC values.
As a tendency towards higher efficacy with increasing concentration was detected, the larvae were treated only with the highest virus concentration of 6.15E+08 OBs/ml in the following experiments.

In general, the standard deviation of the mean efficacies was often very high. As a result, even isolates with enormous differences in their mean efficacy differed not significantly from each other. Variance within the variants of *Tuab*GV La Molina was particularly high during the first experiment. However, this observation did not recur in the second experiment.
Such a high variability might arouse the concern of unreliable control of the pest in the field, which would be an unwanted effect when using an insecticide. However, other GV preparations are already successfully established as biopesticides. Moreover, larvae only took up small amounts of the virus during the hatch, whereas under field conditions the amount taken up by feeding on treated plants would be much higher probably resulting in a higher efficacy in controlling the pest.
In the egg dip assay, larvae took up the virus by feeding on the treated chorion during their hatching process. Although Entwistle & Evans (1985) claimed that the egg dip method provides results of high precision, variability of the mean efficacies may occur as result of the variable uptake of chorion and therefore ingestion of different amounts of occlusion bodies.
In general with a higher number of repetitions the variance might stay high, but the results may become clearer and statistically more reliable.

6.4 Susceptibility of the larval instars

Previous studies have shown that larval susceptibility for virus infection changes with larval age. Usually early instars are more susceptible than later instars (Teakle et al. 1986; Sait et al. 1994; Jeyarani & Karuppuchamy 2010). Accordingly, a decrease in efficacy from the first to the fourth instar would have been expected. A corresponding tendency was observed for *Phop*GV Huancayo. However, the mean efficacies of all four instars did not differ significantly from another.
The third instar was more susceptible to the virus than the second instar. During previous assessments it could be observed that first instar larvae did not leave the leaf tissue for moulting. Therefore second instar larvae did not necessarily come into contact with the treated leaf surface. From the third instar on, larvae were observed to move outside the galleries. This behaviour may explain the higher susceptibility of third instar larvae. In accordance with the expectations, forth instar larvae, were less susceptible than third instar larvae.

The surprisingly low efficacy of *Tuab*GV against the first instar larvae is difficult to explain, especially since *Tuab*GV showed a rather high efficacy in the previous experiments and L1 larvae had to feed on the treated leaves to mine into the plant tissue. A repetition of the experiment would be necessary to re-examine the extreme difference between the efficacy of *Phop*GV Huancayo and *Tuab*GV La Molina against L1 larvae.

6.5 High viral persistence under laboratory conditions

Usually baculoviruses have a rather low persistence. According to Moscardi et al. (2011) half-lifes typically range between 2 and 5 days. In the test of the virus stability, *T. absoluta* was exposed to the virus up to seven days after the virus application. A severe reduction of activity over the time of seven days would have been expected. Kroschel et al. (1996) for example, found a half-life of 1.3 days for *Phop*GV in the Republic of Yemen. On the contrary, during the stability tests in this study, significant loss of pathogenicity over seven days could not be detected for any of the isolates.

The persistence was visible through the consistent efficacy, as well as the infection and pupation rate, which were both unaffected by time either. Both isolates showed an equally high infection rate of nearly 100%, leading to the assumption that the isolates remain biologically active for at least seven days. The high infection rate shows that the virus indeed is biologically active in the *T. absoluta* larvae, but higher concentrations seem to be required for an effective control of the population.
Baculoviruses are known to supress moulting and pupation of larvae (Bonning 2005; Mascarin et al. 2010). Correspondingly, pupation rate of larvae on the virus treated plants was nearly supressed completely (0.00-7.69%). Infected larvae were likely unable to pupate or had died before reaching the pupal stage. On untreated plants in comparison 92.64-98.07% of the larvae were able to pupate.

The consistent efficacy of both virus isolates indicates a high persistence of the virus, which would be an extremely favourable trait for practical use. However, as already elaborated in the discussion of methodology, the stability test was carried out in the laboratory. Therefore UV radiation was probably not comparable to natural conditions on the field and the results are not applicable to the practical use under field conditions. This explains the unusual high persistence of the virus isolates over such a long time. Thus, the stability of the virus should be tested further under outdoor conditions, either in the greenhouse or directly on the field.

7 CONCLUSIONS

This study was conducted to find the *Phop*GV and *Tuab*GV isolates with the highest virulence against *T. absoluta*.
*Phop*GV Huancayo, *Tuab*GV La Molina, and *Tuab*GV St. Rita (Arequipa) provided the best efficacy against *T. absoluta* in the egg dip assay. The first two isolates were further tested in subsequent experiments. Results suggested no significant influence of the larval age on the efficacy, as well a high persistence of the virus on the treated tomato plant.

The study was limited to GV isolates from South America. Isolates from different regions (e.g. from Africa) are available at the CIP laboratories. Since virulence of baculoviruses may vary between isolates of different geographical origins, as it is shown in this study and was also discovered e.g. for *Phop*GV isolates against *P. operculella* (Sporleder 2003), it would be interesting to extend the research to other *Phop*GV and *Tuab*GV isolates.
Viral activity was assessed against one *T. absoluta* population from La Molina, Peru. It is suspected that host species from different geographical locations are likely to vary in their susceptibility to a particular baculovirus (Fuxa 1987; Bonning 2005). Efficient isolates from this study are promising for an efficient *T. absoluta* control in South America. Due to the wide spread of *T. absoluta*, these isolates, as well as those of other origins, should be tested further against local host populations in other countries.
For a successful implementation of *Phop*GV or *Tuab*GV isolates as biological insecticides against *T. absoluta*, the control effect has to be similar to chemical insecticides. Although efficacies of 100% could be achieved in several variants, the probit analysis showed that higher concentrations are required to kill 95% and 99% of the population respectively. Further tests with a wider range of concentrations, especially including concentrations higher than 6.15E+08 OBs/ml, are necessary to verify the theoretical LC values.
Subsequent examination of the two isolates *Phop*GV Huancayo and *Tuab*GV La Molina revealed similar susceptibility of all larval instars against the virus treatment.
Furthermore, consistent biological activity of the virus over seven days after application indicated a high stability of the virus.

In summary, the findings suggest either *Phop*GV as well as *Tuab*GV as potential biological insecticide against *T. absoluta*, whereas the selection of suitable isolates was proved to be an important factor. However, the research on the *T. absoluta* granulovirus as potential biological insecticide against *T. absoluta* has just begun recently. The findings of this study may contribute to the development of alternative control strategies against *T. absoluta*.
Since all experiments were conducted in the laboratory under controlled conditions, promising isolates require further examination under more practical conditions, especially regarding the stability, to see if the findings can be verified under field conditions.

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10 ANNEX

Table A1: Current distribution of *Tuta absoluta* according to EPPO listing.

CONTINENT	COUNTRY	STATE	STATUS
Africa	Algeria		Present, restricted distribution
Africa	Egypt		Absent, unreliable record
Africa	Ethiopia		Present, no details
Africa	Kenya		Present, no details
Africa	Libya		Present, no details
Africa	Morocco		Present, widespread
Africa	Niger		Present, restricted distribution
Africa	Nigeria		Present, no details
Africa	Senegal		Present, no details
Africa	Sudan		Present, restricted distribution
Africa	Tanzania		Present, restricted distribution
Africa	Tunisia		Present, widespread
America	Argentina		Present, widespread
America	Bolivia		Present, widespread
America	Brazil		Present, widespread
America	Brazil	Bahia	Present, no details
America	Brazil	Ceara	Present, no details
America	Brazil	Distrito Federal	Present, no details
America	Brazil	Espirito Santo	Present, no details
America	Brazil	Goias	Present, no details
America	Brazil	Mato Grosso	Present, no details
America	Brazil	Minas Gerais	Present, no details
America	Brazil	Parana	Present, no details
America	Brazil	Pernambuco	Present, no details
America	Brazil	Rio de Janeiro	Present, no details
America	Brazil	Rio Grande do Sul	Present, no details
America	Brazil	Santa Catarina	Present, no details
America	Brazil	Sao Paulo	Present, no details
America	Chile		Present, widespread
America	Colombia		Present, widespread

America	Costa Rica		Present, widespread
America	Ecuador		Present, no details
America	Panama		Present, restricted distribution
America	Paraguay		Present, no details
America	Peru		Present, widespread
America	Uruguay		Present, no details
America	Venezuela		Present, widespread
Asia	Bahrain		Absent, unreliable record
Asia	India		Present, restricted distribution
Asia	India	Karnataka	Present, restricted distribution
Asia	India	Maharashtra	Present, restricted distribution
Asia	Iran		Present, no details
Asia	Iraq		Present, restricted distribution
Asia	Israel		Present, no details
Asia	Japan		Absent, pest no longer present
Asia	Jordan		Present, widespread
Asia	Kuwait		Absent, unreliable record
Asia	Lebanon		Absent, unreliable record
Asia	Qatar		Present, few occurrences
Asia	Saudi Arabia		Present, restricted distribution
Asia	Syria		Present, restricted distribution
Asia	United Arab Emirates		Present, restricted distribution
Asia	Yemen		Present, restricted distribution
Europe	Albania		Present, restricted distribution
Europe	Austria		Transient, under eradication
Europe	Belarus		Present, no details
Europe	Belgium		Present, restricted distribution
Europe	Bosnia and Herzegovina		Present, restricted distribution
Europe	Bulgaria		Present, restricted distribution
Europe	Croatia		Present, restricted distribution
Europe	Cyprus		Present, widespread

Europe	Czech Republic		Transient, under eradication
Europe	Denmark		Absent, intercepted only
Europe	France		Present, restricted distribution
Europe	France	Corse	Present, few occurrences
Europe	Georgia		Present, no details
Europe	Germany		Transient, under eradication
Europe	Greece		Present, restricted distribution
Europe	Greece	Kriti	Present, restricted distribution
Europe	Guernsey		Present, few occurrences
Europe	Hungary		Present, restricted distribution
Europe	Italy		Present, widespread
Europe	Italy	Sardegna	Present, widespread
Europe	Italy	Sicilia	Present, no details
Europe	Lithuania		Present, few occurrences
Europe	Malta		Present, restricted distribution
Europe	Montenegro		Present, no details
Europe	Netherlands		Present, restricted distribution
Europe	Portugal		Present, restricted distribution
Europe	Romania		Present, no details
Europe	Russia		Present, restricted distribution
Europe	Russia	Southern Russia	Present, restricted distribution
Europe	Serbia		Present, restricted distribution
Europe	Slovenia		Present, restricted distribution
Europe	Spain		Present, widespread
Europe	Spain	Islas Baleares	Present, restricted distribution
Europe	Spain	Islas Canárias	Present, no details
Europe	Switzerland		Present, restricted distribution
Europe	Turkey		Present, restricted distribution
Europe	Ukraine		Transient, under eradication
Europe	United Kingdom		Absent, pest eradicated
Europe	United Kingdom	England	Absent, pest eradicated

List according to EPPO (EPPO 2016a).

Table A2: Pairwise Wilcoxon test: Efficacy by isolate 5 dpt, 2.50E+05 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Phop*GV Cuzco	*Phop*GV Chile	4.55024	<.0001*
*Phop*GV La Molina	*Phop*GV Chile	4.67410	<.0001*
*Tuab*GV La Molina	*Phop*GV Chile	4.60406	<.0001*
*Tuab*GV La Molina	*Phop*GV La Molina	3.89129	<.0001*
*Phop*GV La Molina	*Phop*GV Huancayo	3.00547	0.0027*
*Tuab*GV La Molina	*Phop*GV Huancayo	2.96322	0.0030*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	3.00547	0.0027*
*Tuab*GV La Molina	*Phop*GV Huaraz	1.92777	0.0539
*Phop*GV La Molina	*Phop*GV Huaraz	1.64401	0.1002
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	1.64401	0.1002
*Phop*GV Huaraz	*Phop*GV Huancayo	0.57007	0.5686
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	0.91667	0.3593
*Tuab*GV La Molina	*Phop*GV Cuzco	-0.44791	0.6542
*Phop*GV Huaraz	*Phop*GV Chile	-1.48266	0.1382
*Phop*GV Huaraz	*Phop*GV Cuzco	-2.14959	0.0316*
*Phop*GV Huancayo	*Phop*GV Chile	-3.05864	0.0022*
*Phop*GV Huancayo	*Phop*GV Cuzco	-2.99237	0.0028*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	-3.88421	0.0001*
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-3.89129	<.0001*
*Phop*GV La Molina	*Phop*GV Cuzco	-3.90990	<.0001*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	-3.90990	<.0001*

* significant difference at a probability level of $P \leq 0.05$.

Table A3: Pairwise Wilcoxon test: Efficacy by isolate 5 dpt, 5.00E+06 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Phop*GV Cuzco	*Phop*GV Chile	4.76253	<.0001*
*Phop*GV La Molina	*Phop*GV Chile	4.60530	<.0001*
*Tuab*GV La Molina	*Phop*GV Chile	4.45929	<.0001*
*Tuab*GV La Molina	*Phop*GV La Molina	3.60283	0.0003*
*Tuab*GV La Molina	*Phop*GV Huaraz	3.20521	0.0013*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	2.81296	0.0049*
*Phop*GV La Molina	*Phop*GV Huaraz	2.43769	0.0148*
*Tuab*GV La Molina	*Phop*GV Cuzco	2.70241	0.0069*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	2.08923	0.0367*
*Tuab*GV La Molina	*Phop*GV Huancayo	0.90710	0.3644
*Phop*GV Huancayo	*Phop*GV Chile	0.36763	0.7132
*Phop*GV Huancayo	*Phop*GV Cuzco	0.36763	0.7132
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	0.28451	0.7760

Isolate	vs. Isolate	Z	p-value
*Phop*GV La Molina	*Phop*GV Huancayo	-0.35541	0.7223
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	-1.48113	0.1386
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	-1.55943	0.1189
*Phop*GV Huaraz	*Phop*GV Huancayo	-1.89098	0.0586
*Phop*GV Huaraz	*Phop*GV Chile	-2.25921	0.0239*
*Phop*GV Huaraz	*Phop*GV Cuzco	-2.25921	0.0239*
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-2.10864	0.0350*
*Phop*GV La Molina	*Phop*GV Cuzco	-3.05947	0.0022*

* significant difference at a probability level of $P \leq 0.05$.

Table A4: Pairwise Wilcoxon test: Efficacy by isolate 5 dpt, 7.50E+07 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Phop*GV La Molina	*Phop*GV Chile	4.60406	<.0001*
*Tuab*GV La Molina	*Phop*GV Chile	4.45478	<.0001*
*Phop*GV Cuzco	*Phop*GV Chile	4.57278	<.0001*
*Tuab*GV La Molina	*Phop*GV La Molina	3.59853	0.0003*
*Phop*GV La Molina	*Phop*GV Huaraz	3.59915	0.0003*
*Tuab*GV La Molina	*Phop*GV Huaraz	3.47233	0.0005*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	3.49938	0.0005*
*Tuab*GV La Molina	*Phop*GV Cuzco	2.12267	0.0338*
*Tuab*GV La Molina	*Phop*GV Huancayo	1.61461	0.1064
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	1.51958	0.1286
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	0.64897	0.5164
*Phop*GV La Molina	*Phop*GV Huancayo	0.45338	0.6503
*Phop*GV Huancayo	*Phop*GV Chile	0.00000	1.0000
*Phop*GV Huancayo	*Phop*GV Cuzco	-0.22704	0.8204
*Phop*GV Huaraz	*Phop*GV Huancayo	-1.77621	0.0757
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	-2.57647	0.0100*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	-2.59616	0.0094*
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-2.83671	0.0046*
*Phop*GV La Molina	*Phop*GV Cuzco	-3.06096	0.0022*
*Phop*GV Huaraz	*Phop*GV Cuzco	-3.56704	0.0004*
*Phop*GV Huaraz	*Phop*GV Chile	-3.73502	0.0002*

* significant difference at a probability level of $P \leq 0.05$.

Table A5: Pairwise Wilcoxon test: Efficacy by isolate 5 dpt, 3.00E+08 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV La Molina	*Phop*GV Chile	4.03163	<.0001*
*Tuab*GV La Molina	*Phop*GV La Molina	4.03163	<.0001*
*Phop*GV Cuzco	*Phop*GV Chile	3.89129	<.0001*
*Phop*GV La Molina	*Phop*GV Chile	3.88421	0.0001*
*Tuab*GV La Molina	*Phop*GV Huaraz	3.39093	0.0007*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	2.83991	0.0045*
*Phop*GV La Molina	*Phop*GV Huaraz	2.64438	0.0082*
*Tuab*GV La Molina	*Phop*GV Cuzco	1.85954	0.0630
*Phop*GV Huancayo	*Phop*GV Chile	1.07984	0.2802
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	1.11244	0.2660
*Phop*GV Huancayo	*Phop*GV Cuzco	0.67685	0.4985
*Tuab*GV La Molina	*Phop*GV Huancayo	0.00000	1.0000
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	-0.79634	0.4258
*Phop*GV La Molina	*Phop*GV Huancayo	-1.07984	0.2802
*Phop*GV Huaraz	*Phop*GV Huancayo	-2.22936	0.0258*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	-2.28338	0.0224*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	-2.43708	0.0148*
*Phop*GV Huaraz	*Phop*GV Chile	-2.64438	0.0082*
*Phop*GV Huaraz	*Phop*GV Cuzco	-2.80954	0.0050*
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-2.77567	0.0055*
*Phop*GV La Molina	*Phop*GV Cuzco	-3.89129	<.0001*

* significant difference at a probability level of $P \leq 0.05$.

Table A6: Pairwise Wilcoxon test: Efficacy by isolate 5 dpt, 6.15E+08 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Phop*GV Cuzco	*Phop*GV Chile	3.88964	0.0001*
*Phop*GV La Molina	*Phop*GV Chile	3.66650	0.0002*
*Tuab*GV La Molina	*Phop*GV Chile	3.56576	0.0004*
*Tuab*GV La Molina	*Phop*GV Cuzco	3.75416	0.0002*
*Tuab*GV La Molina	*Phop*GV Huaraz	2.64460	0.0082*
*Tuab*GV La Molina	*Phop*GV La Molina	2.47676	0.0133*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	2.53453	0.0113*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	1.83474	0.0665
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	1.59360	0.1110
*Tuab*GV La Molina	*Phop*GV Huancayo	1.52676	0.1268
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	1.16599	0.2436
*Phop*GV La Molina	*Phop*GV Huancayo	0.78621	0.4317
*Phop*GV Huaraz	*Phop*GV Cuzco	0.71506	0.4746

Isolate	vs. Isolate	Z	p-value
*Phop*GV La Molina	*Phop*GV Huaraz	0.67764	0.4980
*Phop*GV Huancayo	*Phop*GV Cuzco	0.36795	0.7129
*Phop*GV Huaraz	*Phop*GV Chile	0.27526	0.7831
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	0.21935	0.8264
*Phop*GV Huancayo	*Phop*GV Chile	0.03282	0.9738
*Phop*GV Huaraz	*Phop*GV Huancayo	-0.06524	0.9480
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-0.33057	0.7410
*Phop*GV La Molina	*Phop*GV Cuzco	-0.72053	0.4712

* significant difference at a probability level of $P \leq 0.05$.

Table A7: Pairwise Wilcoxon test: Efficacy by OBs/ml 5 dpt, *Phop*GV Chile

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	2.50E+05	0.9166667	0.3593
3.00E+08	5.00E+06	0.9166667	0.3593
3.00E+08	7.50E+07	0.9166667	0.3593
6.15E+08	2.50E+05	0.9166667	0.3593
6.15E+08	5.00E+06	0.9166667	0.3593
6.15E+08	7.50E+07	0.9166667	0.3593
5.00E+06	2.50E+05	.	.
7.50E+07	2.50E+05	.	.
7.50E+07	5.00E+06	.	.
6.15E+08	3.00E+08	0.0000000	1.0000

Significant difference at a probability level of $P \leq 0.05$.

Table A8: Pairwise Wilcoxon test: Efficacy by OBs/ml 5 dpt, *Phop*GV Cuzco

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	5.00E+06	1.38444	0.1662
7.50E+07	5.00E+06	0.95743	0.3384
3.00E+08	7.50E+07	0.57808	0.5632
6.15E+08	5.00E+06	.	.
3.00E+08	2.50E+05	-0.36599	0.7144
6.15E+08	7.50E+07	-0.95743	0.3384
7.50E+07	2.50E+05	-0.98006	0.3271
6.15E+08	3.00E+08	-1.38444	0.1662
5.00E+06	2.50E+05	-1.75882	0.0786
6.15E+08	2.50E+05	-1.75882	0.0786

Significant difference at a probability level of $P \leq 0.05$.

Table A9: Pairwise Wilcoxon test: Efficacy by OBs/ml 5 dpt, *Phop*GV Huancayo

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	2.50E+05	1.96353	0.0496*
3.00E+08	7.50E+07	1.48303	0.1381
6.15E+08	2.50E+05	1.48604	0.1373
5.00E+06	2.50E+05	1.41210	0.1579
7.50E+07	2.50E+05	1.23943	0.2152
3.00E+08	5.00E+06	0.81835	0.4132
6.15E+08	7.50E+07	0.62312	0.5332
6.15E+08	5.00E+06	-0.17293	0.8627
7.50E+07	5.00E+06	-0.81647	0.4142
6.15E+08	3.00E+08	-0.95678	0.3387

* significant difference at a probability level of $P \leq 0.05$.

Table A10: Pairwise Wilcoxon test: Efficacy by OBs/ml 5 dpt, *Phop*GV Huaraz

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	7.50E+07	2.07021	0.0384*
6.15E+08	5.00E+06	1.62886	0.1033
6.15E+08	3.00E+08	1.67887	0.0932
6.15E+08	2.50E+05	1.31051	0.1900
3.00E+08	7.50E+07	0.52040	0.6028
3.00E+08	5.00E+06	-0.18006	0.8571
5.00E+06	2.50E+05	-0.36195	0.7174
3.00E+08	2.50E+05	-0.54884	0.5831
7.50E+07	5.00E+06	-0.79203	0.4283
7.50E+07	2.50E+05	-1.15302	0.2489

* significant difference at a probability level of $P \leq 0.05$.

Table A11: Pairwise Wilcoxon test: Efficacy by OBs/ml 5 dpt, *Phop*GV La Molina

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	2.50E+05	1.86716	0.0619
6.15E+08	3.00E+08	1.86716	0.0619
6.15E+08	7.50E+07	1.51503	0.1298
6.15E+08	5.00E+06	1.41161	0.1581
5.00E+06	2.50E+05	0.55387	0.5797
7.50E+07	2.50E+05	0.50252	0.6153
3.00E+08	2.50E+05	0.00000	1.0000
7.50E+07	5.00E+06	-0.04454	0.9645
3.00E+08	7.50E+07	-0.50252	0.6153
3.00E+08	5.00E+06	-0.55387	0.5797

Significant difference at a probability level of $P \leq 0.05$.

Table A12: Pairwise Wilcoxon test: Efficacy by OBs/ml 5 dpt, *Tuab*GV La Molina

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	2.50E+05	2.843240	0.0045*
3.00E+08	2.50E+05	2.145742	0.0319*
5.00E+06	2.50E+05	1.727311	0.0841
7.50E+07	2.50E+05	1.691186	0.0908
6.15E+08	7.50E+07	1.086187	0.2774
6.15E+08	5.00E+06	1.023930	0.3059
6.15E+08	3.00E+08	0.783297	0.4335
3.00E+08	5.00E+06	0.457732	0.6471
3.00E+08	7.50E+07	0.424092	0.6715
7.50E+07	5.00E+06	0.000000	1.0000

* significant difference at a probability level of $P \leq 0.05$.

Table A13: Pairwise Wilcoxon test: Efficacy by OBs/ml 5 dpt, *Tuab*GV St. Rita (Arequipa)

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	2.50E+05	2.75178	0.0059*
6.15E+08	3.00E+08	1.83823	0.0660
6.15E+08	7.50E+07	1.79842	0.0721
6.15E+08	5.00E+06	1.52935	0.1262
5.00E+06	2.50E+05	1.54531	0.1223
3.00E+08	2.50E+05	1.11244	0.2660
7.50E+07	2.50E+05	0.77388	0.4390
3.00E+08	7.50E+07	0.17977	0.8573
3.00E+08	5.00E+06	-0.35962	0.7191
7.50E+07	5.00E+06	-0.58834	0.5563

* significant difference at a probability level of $P \leq 0.05$.

Table A14: Pairwise Wilcoxon test: Efficacy by isolate 10 dpt, 2.50E+05 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Phop*GV Cuzco	*Phop*GV Chile	4.55024	<.0001*
*Tuab*GV La Molina	*Phop*GV Chile	4.42345	<.0001*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	3.39406	0.0007*
*Tuab*GV La Molina	*Phop*GV La Molina	3.09827	0.0019*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	2.02508	0.0429*
*Phop*GV La Molina	*Phop*GV Huancayo	1.93877	0.0525
*Tuab*GV La Molina	*Phop*GV Huancayo	1.91649	0.0553
*Phop*GV Huaraz	*Phop*GV Huancayo	0.95460	0.3398
*Phop*GV Huaraz	*Phop*GV Chile	0.71398	0.4752
*Tuab*GV La Molina	*Phop*GV Cuzco	0.37905	0.7047

Isolate	vs. Isolate	Z	p-value
*Phop*GV Huaraz	*Phop*GV Cuzco	0.14915	0.8814
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	0.02958	0.9764
*Tuab*GV La Molina	*Phop*GV Huaraz	-0.03142	0.9749
*Phop*GV La Molina	*Phop*GV Huaraz	-0.57265	0.5669
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	-1.48151	0.1385
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-1.62755	0.1036
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	-1.66398	0.0961
*Phop*GV Huancayo	*Phop*GV Chile	-2.00843	0.0446*
*Phop*GV Huancayo	*Phop*GV Cuzco	-1.98010	0.0477*
*Phop*GV La Molina	*Phop*GV Chile	-3.05864	0.0022*
*Phop*GV La Molina	*Phop*GV Cuzco	-3.17747	0.0015*

* significant difference at a probability level of $P \leq 0.05$.

Table A15: Pairwise Wilcoxon test: Efficacy by isolate 10 dpt, 5.00E+06 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV La Molina	*Phop*GV Chile	3.93869	<.0001*
*Phop*GV Cuzco	*Phop*GV Chile	3.88421	0.0001*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	2.80916	0.0050*
*Tuab*GV La Molina	*Phop*GV La Molina	2.69211	0.0071*
*Tuab*GV La Molina	*Phop*GV Huaraz	2.57319	0.0101*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	2.45376	0.0141*
*Tuab*GV La Molina	*Phop*GV Cuzco	2.47962	0.0132*
*Phop*GV Huancayo	*Phop*GV Chile	1.86155	0.0627
*Phop*GV Huancayo	*Phop*GV Cuzco	1.86155	0.0627
*Phop*GV La Molina	*Phop*GV Huaraz	1.65024	0.0989
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	0.57983	0.5620
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	0.51880	0.6039
*Tuab*GV La Molina	*Phop*GV Huancayo	-0.09339	0.9256
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	-0.89955	0.3684
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-0.99278	0.3208
*Phop*GV Huaraz	*Phop*GV Chile	-1.58078	0.1139
*Phop*GV Huaraz	*Phop*GV Cuzco	-1.64277	0.1004
*Phop*GV La Molina	*Phop*GV Chile	-1.70519	0.0882
*Phop*GV La Molina	*Phop*GV Cuzco	-1.70519	0.0882
*Phop*GV La Molina	*Phop*GV Huancayo	-1.73279	0.0831
*Phop*GV Huaraz	*Phop*GV Huancayo	-2.45145	0.0142*

* significant difference at a probability level of $P \leq 0.05$.

Table A16: Pairwise Wilcoxon test: Efficacy by isolate 10 dpt, 7.50E+07 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV La Molina	*Phop*GV La Molina	3.70800	0.0002*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	3.20901	0.0013*
*Tuab*GV La Molina	*Phop*GV Chile	2.07918	0.0376*
*Tuab*GV La Molina	*Phop*GV Cuzco	2.31120	0.0208*
*Tuab*GV La Molina	*Phop*GV Huaraz	1.96160	0.0498*
*Phop*GV Huancayo	*Phop*GV Cuzco	0.98694	0.3237
*Tuab*GV La Molina	*Phop*GV Huancayo	0.87424	0.3820
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	0.75911	0.4478
*Phop*GV Huancayo	*Phop*GV Chile	0.02920	0.9767
*Phop*GV Cuzco	*Phop*GV Chile	0.00000	1.0000
*Phop*GV La Molina	*Phop*GV Huaraz	-0.09693	0.9228
*Phop*GV Huaraz	*Phop*GV Cuzco	-0.13698	0.8910
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	-0.30268	0.7621
*Phop*GV Huaraz	*Phop*GV Huancayo	-0.89213	0.3723
*Phop*GV La Molina	*Phop*GV Huancayo	-1.17210	0.2412
*Phop*GV Huaraz	*Phop*GV Chile	-1.28147	0.2000
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	-1.60988	0.1074
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	-2.00074	0.0454*
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-2.73455	0.0062*
*Phop*GV La Molina	*Phop*GV Cuzco	-3.15571	0.0016*
*Phop*GV La Molina	*Phop*GV Chile	-3.60024	0.0003*

* significant difference at a probability level of $P \leq 0.05$.

Table A17: Pairwise Wilcoxon test: Efficacy by isolate 10 dpt, 3.00E+08 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV La Molina	*Phop*GV La Molina	3.50330	0.0005*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	3.14417	0.0017*
*Tuab*GV La Molina	*Phop*GV Huaraz	3.09363	0.0020*
*Phop*GV La Molina	*Phop*GV Huaraz	2.66816	0.0076*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	2.78497	0.0054*
*Tuab*GV La Molina	*Phop*GV Cuzco	1.78004	0.0751
*Phop*GV Huancayo	*Phop*GV Chile	1.10565	0.2689
*Tuab*GV La Molina	*Phop*GV Chile	1.12701	0.2597
*Phop*GV Huancayo	*Phop*GV Cuzco	1.02183	0.3069
*Phop*GV Cuzco	*Phop*GV Chile	-0.29086	0.7712
*Tuab*GV La Molina	*Phop*GV Huancayo	-0.39902	0.6899
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	-0.87433	0.3819
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	-0.86525	0.3869
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	-1.36498	0.1723

Isolate	vs. Isolate	Z	p-value
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-1.48741	0.1369
*Phop*GV La Molina	*Phop*GV Huancayo	-1.83545	0.0664
*Phop*GV Huaraz	*Phop*GV Cuzco	-2.66026	0.0078*
*Phop*GV Huaraz	*Phop*GV Huancayo	-2.87031	0.0041*
*Phop*GV La Molina	*Phop*GV Cuzco	-3.03321	0.0024*
*Phop*GV Huaraz	*Phop*GV Chile	-3.08588	0.0020*
*Phop*GV La Molina	*Phop*GV Chile	-3.33520	0.0009*

* significant difference at a probability level of $P \leq 0.05$.

Table A18: Pairwise Wilcoxon test: Efficacy by isolate 10 dpt, 6.15E+08 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV La Molina	*Phop*GV Cuzco	3.22067	0.0013*
*Tuab*GV La Molina	*Phop*GV La Molina	2.62992	0.0085*
*Tuab*GV La Molina	*Phop*GV Chile	2.56482	0.0103*
*Tuab*GV La Molina	*Phop*GV Huaraz	2.24009	0.0251*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	2.07932	0.0376*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	1.78817	0.0737
*Phop*GV Huancayo	*Phop*GV Cuzco	1.59139	0.1115
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	1.59668	0.1103
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	1.27306	0.2030
*Phop*GV La Molina	*Phop*GV Cuzco	1.18212	0.2372
*Tuab*GV La Molina	*Phop*GV Huancayo	1.10667	0.2684
*Phop*GV Huaraz	*Phop*GV Cuzco	0.94236	0.3460
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	0.79543	0.4264
*Phop*GV Huancayo	*Phop*GV Chile	0.77509	0.4383
*Phop*GV Huaraz	*Phop*GV Chile	-0.03110	0.9752
*Phop*GV La Molina	*Phop*GV Huaraz	-0.21757	0.8278
*Phop*GV Cuzco	*Phop*GV Chile	-0.56970	0.5689
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-0.61181	0.5407
*Phop*GV La Molina	*Phop*GV Huancayo	-0.83667	0.4028
*Phop*GV Huaraz	*Phop*GV Huancayo	-0.90374	0.3661
*Phop*GV La Molina	*Phop*GV Chile	-0.90398	0.3660

* significant difference at a probability level of $P \leq 0.05$.

Table A19: Pairwise Wilcoxon test: Efficacy by OBs/ml 10 dpt, *Phop*GV Chile

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	2.50E+05	3.26970	0.0011*
3.00E+08	5.00E+06	2.85675	0.0043*
6.15E+08	2.50E+05	2.98690	0.0028*
6.15E+08	5.00E+06	2.47962	0.0132*
7.50E+07	2.50E+05	2.69935	0.0069*
7.50E+07	5.00E+06	2.23647	0.0253*
3.00E+08	7.50E+07	0.75152	0.4523
5.00E+06	2.50E+05	0.91667	0.3593
6.15E+08	7.50E+07	0.24343	0.8077
6.15E+08	3.00E+08	-0.59646	0.5509

* significant difference at a probability level of $P \leq 0.05$.

Table A20: Pairwise Wilcoxon test: Efficacy by OBs/ml 10 dpt, *Phop*GV Cuzco

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	5.00E+06	0.93368	0.3505
7.50E+07	5.00E+06	0.52590	0.5990
3.00E+08	7.50E+07	0.38066	0.7035
3.00E+08	2.50E+05	0.00000	1.0000
6.15E+08	5.00E+06	-0.65465	0.5127
7.50E+07	2.50E+05	-0.42827	0.6685
6.15E+08	7.50E+07	-1.07981	0.2802
6.15E+08	3.00E+08	-1.19611	0.2317
5.00E+06	2.50E+05	-0.97844	0.3279
6.15E+08	2.50E+05	-1.33193	0.1829

Significant difference at a probability level of $P \leq 0.05$.

Table A21: Pairwise Wilcoxon test: Efficacy by OBs/ml 10 dpt, *Phop*GV Huancayo

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	2.50E+05	2.12815	0.0333*
6.15E+08	2.50E+05	1.89698	0.0578
5.00E+06	2.50E+05	1.86185	0.0626
7.50E+07	2.50E+05	1.39717	0.1624
3.00E+08	7.50E+07	1.14905	0.2505
6.15E+08	7.50E+07	0.75166	0.4523
3.00E+08	5.00E+06	0.03150	0.9749
6.15E+08	5.00E+06	-0.16697	0.8674
6.15E+08	3.00E+08	-0.28340	0.7769
7.50E+07	5.00E+06	-0.87716	0.3804

* significant difference at a probability level of $P \leq 0.05$.

Table A22: Pairwise Wilcoxon test: Efficacy by OBs/ml 10 dpt, *Phop*GV Huaraz

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	3.00E+08	1.84268	0.0654
6.15E+08	5.00E+06	1.53869	0.1239
6.15E+08	7.50E+07	0.85689	0.3915
7.50E+07	5.00E+06	0.68206	0.4952
6.15E+08	2.50E+05	0.31887	0.7498
3.00E+08	5.00E+06	-0.54732	0.5842
7.50E+07	2.50E+05	-0.67722	0.4983
3.00E+08	7.50E+07	-1.25222	0.2105
5.00E+06	2.50E+05	-1.32666	0.1846
3.00E+08	2.50E+05	-1.82612	0.0678

Significant difference at a probability level of $P \leq 0.05$.

Table A23: Pairwise Wilcoxon test: Efficacy by OBs/ml 10 dpt, *Phop*GV La Molina

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	7.50E+07	2.57218	0.0101*
6.15E+08	2.50E+05	2.36897	0.0178*
6.15E+08	3.00E+08	2.10359	0.0354*
6.15E+08	5.00E+06	1.59159	0.1115
5.00E+06	2.50E+05	0.87543	0.3813
3.00E+08	7.50E+07	0.13342	0.8939
3.00E+08	2.50E+05	0.08895	0.9291
7.50E+07	2.50E+05	-0.04454	0.9645
3.00E+08	5.00E+06	-0.64632	0.5181
7.50E+07	5.00E+06	-1.02885	0.3036

* significant difference at a probability level of $P \leq 0.05$.

Table A24: Pairwise Wilcoxon test: Efficacy by OBs/ml 10 dpt, *Tuab*GV La Molina

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	2.50E+05	3.061711	0.0022*
3.00E+08	2.50E+05	2.432871	0.0150*
7.50E+07	2.50E+05	1.630500	0.1030
6.15E+08	5.00E+06	1.524619	0.1274
5.00E+06	2.50E+05	1.431674	0.1522
6.15E+08	3.00E+08	1.309938	0.1902
6.15E+08	7.50E+07	1.223715	0.2211
3.00E+08	5.00E+06	0.572732	0.5668
7.50E+07	5.00E+06	0.211527	0.8325
3.00E+08	7.50E+07	0.101250	0.9194

* significant difference at a probability level of $P \leq 0.05$.

Table A25: Pairwise Wilcoxon test: Efficacy by OBs/ml 10 dpt, *Tuab*GV St. Rita (Arequipa)

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	2.50E+05	2.55307	0.0107*
6.15E+08	7.50E+07	2.24305	0.0249*
6.15E+08	5.00E+06	1.58473	0.1130
6.15E+08	3.00E+08	1.42088	0.1554
5.00E+06	2.50E+05	1.26086	0.2074
3.00E+08	2.50E+05	0.73349	0.4633
3.00E+08	7.50E+07	0.61499	0.5386
7.50E+07	2.50E+05	0.03995	0.9681
3.00E+08	5.00E+06	-0.30128	0.7632
7.50E+07	5.00E+06	-1.15447	0.2483

* significant difference at a probability level of $P \leq 0.05$.

Table A26: Pairwise Wilcoxon test: Efficacy by isolate 15 dpt, 2.50E+05 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV La Molina	*Phop*GV La Molina	3.17791	0.0015*
*Phop*GV Huaraz	*Phop*GV Cuzco	2.83235	0.0046*
*Phop*GV Huaraz	*Phop*GV Chile	2.44930	0.0143*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	2.50781	0.0121*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	2.25326	0.0242*
*Tuab*GV La Molina	*Phop*GV Cuzco	1.89375	0.0583
*Phop*GV Huaraz	*Phop*GV Huancayo	1.61010	0.1074
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	1.47351	0.1406
*Tuab*GV La Molina	*Phop*GV Chile	1.35262	0.1762
*Tuab*GV La Molina	*Phop*GV Huancayo	1.36160	0.1733
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	1.17393	0.2404
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-0.36541	0.7148
*Phop*GV Huancayo	*Phop*GV Cuzco	-0.59552	0.5515
*Phop*GV Huancayo	*Phop*GV Chile	-0.72974	0.4655
*Phop*GV Cuzco	*Phop*GV Chile	-0.97872	0.3277
*Tuab*GV La Molina	*Phop*GV Huaraz	-0.98939	0.3225
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	-1.37209	0.1700
*Phop*GV La Molina	*Phop*GV Chile	-2.24928	0.0245*
*Phop*GV La Molina	*Phop*GV Cuzco	-2.40287	0.0163*
*Phop*GV La Molina	*Phop*GV Huancayo	-2.50715	0.0122*
*Phop*GV La Molina	*Phop*GV Huaraz	-3.74857	0.0002*

* significant difference at a probability level of $P \leq 0.05$.

Table A27: Pairwise Wilcoxon test: Efficacy by isolate 15 dpt, 5.00E+06 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	3.26252	0.0011*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	3.22159	0.0013*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	2.58225	0.0098*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	2.56486	0.0103*
*Phop*GV Huancayo	*Phop*GV Cuzco	2.34594	0.0190*
*Phop*GV Huancayo	*Phop*GV Chile	1.98273	0.0474*
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	2.12426	0.0336*
*Tuab*GV La Molina	*Phop*GV La Molina	1.48686	0.1371
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	1.19626	0.2316
*Tuab*GV La Molina	*Phop*GV Cuzco	1.11306	0.2657
*Phop*GV Huaraz	*Phop*GV Cuzco	0.49873	0.6180
*Tuab*GV La Molina	*Phop*GV Huaraz	0.49496	0.6206
*Phop*GV Huaraz	*Phop*GV Chile	-0.26146	0.7937
*Tuab*GV La Molina	*Phop*GV Chile	-0.40807	0.6832
*Tuab*GV La Molina	*Phop*GV Huancayo	-0.68692	0.4921
*Phop*GV La Molina	*Phop*GV Cuzco	-0.73441	0.4627
*Phop*GV Cuzco	*Phop*GV Chile	-2.02379	0.0430*
*Phop*GV La Molina	*Phop*GV Chile	-2.00719	0.0447*
*Phop*GV Huaraz	*Phop*GV Huancayo	-2.33583	0.0195*
*Phop*GV La Molina	*Phop*GV Huaraz	-2.35679	0.0184*
*Phop*GV La Molina	*Phop*GV Huancayo	-3.06781	0.0022*

* significant difference at a probability level of $P \leq 0.05$.

Table A28: Pairwise Wilcoxon test: Efficacy by isolate 15 dpt, 7.50E+07 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	4.04829	<.0001*
*Tuab*GV La Molina	*Phop*GV Cuzco	3.64310	0.0003*
*Phop*GV Huancayo	*Phop*GV Cuzco	3.69610	0.0002*
*Phop*GV La Molina	*Phop*GV Cuzco	3.14601	0.0017*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	2.83456	0.0046*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	2.83620	0.0046*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	2.55259	0.0107*
*Phop*GV Huaraz	*Phop*GV Cuzco	1.63520	0.1020
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	1.55893	0.1190
*Phop*GV Huancayo	*Phop*GV Chile	1.45534	0.1456
*Tuab*GV La Molina	*Phop*GV Huaraz	1.45607	0.1454
*Tuab*GV La Molina	*Phop*GV La Molina	1.36182	0.1733
*Tuab*GV La Molina	*Phop*GV Chile	1.01367	0.3107
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	0.87535	0.3814

Isolate	vs. Isolate	Z	p-value
*Tuab*GV La Molina	*Phop*GV Huancayo	-0.28570	0.7751
*Phop*GV La Molina	*Phop*GV Huaraz	-0.33951	0.7342
*Phop*GV La Molina	*Phop*GV Chile	-0.37610	0.7068
*Phop*GV Huaraz	*Phop*GV Chile	-0.58613	0.5578
*Phop*GV Huaraz	*Phop*GV Huancayo	-1.66627	0.0957
*Phop*GV La Molina	*Phop*GV Huancayo	-1.95175	0.0510
*Phop*GV Cuzco	*Phop*GV Chile	-3.71606	0.0002*

* significant difference at a probability level of $P \leq 0.05$.

Table A29: Pairwise Wilcoxon test: Efficacy by isolate 15 dpt, 3.00E+08 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Phop*GV Huancayo	*Phop*GV Cuzco	2.42327	0.0154*
*Phop*GV Huancayo	*Phop*GV Chile	1.73613	0.0825
*Tuab*GV La Molina	*Phop*GV Huaraz	1.27228	0.2033
*Phop*GV La Molina	*Phop*GV Cuzco	1.07874	0.2807
*Phop*GV La Molina	*Phop*GV Huaraz	1.03423	0.3010
*Tuab*GV La Molina	*Phop*GV Cuzco	1.04091	0.2979
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	0.62459	0.5322
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	0.39984	0.6893
*Phop*GV La Molina	*Phop*GV Chile	0.28158	0.7783
*Phop*GV Huaraz	*Phop*GV Cuzco	-0.53565	0.5922
*Tuab*GV La Molina	*Phop*GV Chile	-0.70051	0.4836
*Tuab*GV La Molina	*Phop*GV La Molina	-0.74620	0.4555
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-0.77944	0.4357
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	-1.18380	0.2365
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	-1.26358	0.2064
*Phop*GV La Molina	*Phop*GV Huancayo	-1.48343	0.1380
*Phop*GV Cuzco	*Phop*GV Chile	-1.69786	0.0895
*Phop*GV Huaraz	*Phop*GV Chile	-1.75835	0.0787
*Tuab*GV La Molina	*Phop*GV Huancayo	-2.02198	0.0432*
*Phop*GV Huaraz	*Phop*GV Huancayo	-2.64851	0.0081*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	-2.64483	0.0082*

* significant difference at a probability level of $P \leq 0.05$.

Table A30: Pairwise Wilcoxon test: Efficacy by isolate 15 dpt, 6.15E+08 OBs/ml.

Isolate	vs. Isolate	Z	p-value
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Cuzco	3.64149	0.0003*
*Tuab*GV La Molina	*Phop*GV Cuzco	3.41524	0.0006*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huaraz	2.58737	0.0097*
*Phop*GV La Molina	*Phop*GV Cuzco	2.52937	0.0114*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV La Molina	2.36518	0.0180*
*Tuab*GV La Molina	*Phop*GV Huaraz	2.46475	0.0137*
*Phop*GV Huaraz	*Phop*GV Cuzco	2.36909	0.0178*
*Tuab*GV La Molina	*Phop*GV La Molina	2.23598	0.0254*
*Phop*GV Huancayo	*Phop*GV Cuzco	2.13218	0.0330*
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Chile	0.75671	0.4492
*Tuab*GV La Molina	*Phop*GV Huancayo	0.71865	0.4724
*Tuab*GV St. Rita (Arequipa)	*Phop*GV Huancayo	0.68133	0.4957
*Tuab*GV La Molina	*Phop*GV Chile	0.22017	0.8257
*Tuab*GV St. Rita (Arequipa)	*Tuab*GV La Molina	-0.04062	0.9676
*Phop*GV Huancayo	*Phop*GV Chile	-0.28202	0.7779
*Phop*GV La Molina	*Phop*GV Huaraz	-0.71201	0.4765
*Phop*GV Huaraz	*Phop*GV Huancayo	-1.84856	0.0645
*Phop*GV La Molina	*Phop*GV Huancayo	-2.07932	0.0376*
*Phop*GV La Molina	*Phop*GV Chile	-2.73142	0.0063*
*Phop*GV Huaraz	*Phop*GV Chile	-3.00734	0.0026*
*Phop*GV Cuzco	*Phop*GV Chile	-3.71011	0.0002*

* significant difference at a probability level of $P \leq 0.05$.

Table A31: Pairwise Wilcoxon test: Efficacy by OBs/ml 15 dpt, *Phop*GV Chile

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	2.50E+05	3.926561	<.0001*
6.15E+08	5.00E+06	3.201280	0.0014*
3.00E+08	2.50E+05	2.920611	0.0035*
7.50E+07	2.50E+05	2.491539	0.0127*
6.15E+08	7.50E+07	2.337519	0.0194*
3.00E+08	5.00E+06	1.755487	0.0792
5.00E+06	2.50E+05	1.390285	0.1644
7.50E+07	5.00E+06	1.285063	0.1988
3.00E+08	7.50E+07	0.930870	0.3519
6.15E+08	3.00E+08	0.939200	0.3476

* significant difference at a probability level of $P \leq 0.05$.

Table A32: Pairwise Wilcoxon test: Efficacy by OBs/ml 15 dpt, *Phop*GV Cuzco

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	7.50E+07	1.96630	0.0493*
3.00E+08	2.50E+05	0.94150	0.3465
3.00E+08	5.00E+06	0.96501	0.3345
5.00E+06	2.50E+05	0.00000	1.0000
6.15E+08	7.50E+07	-0.24442	0.8069
6.15E+08	5.00E+06	-1.20390	0.2286
7.50E+07	5.00E+06	-1.32469	0.1853
7.50E+07	2.50E+05	-1.41355	0.1575
6.15E+08	2.50E+05	-1.48304	0.1381
6.15E+08	3.00E+08	-1.79064	0.0734

* significant difference at a probability level of $P \leq 0.05$.

Table A33: Pairwise Wilcoxon test: Efficacy by OBs/ml 15 dpt, *Phop*GV Huancayo

OBs/ml	vs. OBs/ml	Z	p-value
3.00E+08	2.50E+05	3.02288	0.0025*
7.50E+07	2.50E+05	2.38139	0.0172*
6.15E+08	2.50E+05	2.07116	0.0383*
5.00E+06	2.50E+05	1.89340	0.0583
3.00E+08	5.00E+06	1.13550	0.2562
3.00E+08	7.50E+07	0.76867	0.4421
7.50E+07	5.00E+06	0.54000	0.5892
6.15E+08	5.00E+06	0.43693	0.6622
6.15E+08	7.50E+07	-0.20484	0.8377
6.15E+08	3.00E+08	-0.72334	0.4695

* significant difference at a probability level of $P \leq 0.05$.

Table A34: Pairwise Wilcoxon test: Efficacy by OBs/ml 15 dpt, *Phop*GV Huaraz

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	5.00E+06	1.56797	0.1169
7.50E+07	5.00E+06	0.84497	0.3981
6.15E+08	3.00E+08	0.85522	0.3924
6.15E+08	7.50E+07	0.20101	0.8407
3.00E+08	5.00E+06	0.00000	1.0000
6.15E+08	2.50E+05	-0.15806	0.8744
3.00E+08	7.50E+07	-0.42627	0.6699
7.50E+07	2.50E+05	-0.43757	0.6617
3.00E+08	2.50E+05	-1.12364	0.2612
5.00E+06	2.50E+05	-1.68858	0.0913

Significant difference at a probability level of $P \leq 0.05$.

Table A35: Pairwise Wilcoxon test: Efficacy by OBs/ml 15 dpt, *Phop*GV La Molina

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	5.00E+06	3.40726	0.0007*
6.15E+08	2.50E+05	3.45225	0.0006*
7.50E+07	2.50E+05	3.39273	0.0007*
7.50E+07	5.00E+06	3.26599	0.0011*
3.00E+08	2.50E+05	3.12557	0.0018*
3.00E+08	5.00E+06	2.83917	0.0045*
3.00E+08	7.50E+07	1.58877	0.1121
5.00E+06	2.50E+05	1.21494	0.2244
6.15E+08	7.50E+07	0.11688	0.9070
6.15E+08	3.00E+08	-1.52646	0.1269

* significant difference at a probability level of $P \leq 0.05$.

Table A36: Pairwise Wilcoxon test: Efficacy by OBs/ml 15 dpt, *Tuab*GV La Molina

OBs/ml	vs. OBs/ml	Z	p-value
6.15E+08	2.50E+05	2.55165	0.0107*
6.15E+08	5.00E+06	2.06355	0.0391*
7.50E+07	2.50E+05	1.74100	0.0817
6.15E+08	3.00E+08	1.76819	0.0770
7.50E+07	5.00E+06	1.41485	0.1571
3.00E+08	2.50E+05	1.06530	0.2867
6.15E+08	7.50E+07	1.07578	0.2820
3.00E+08	5.00E+06	0.90590	0.3650
5.00E+06	2.50E+05	-0.37583	0.7070
3.00E+08	7.50E+07	-0.66928	0.5033

* significant difference at a probability level of $P \leq 0.05$.

Table A37: Pairwise Wilcoxon test: Efficacy by OBs/ml 15 dpt, *Tuab*GV St. Rita (Arequipa)

OBs/ml	vs. OBs/ml	Z	p-value
7.50E+07	2.50E+05	3.10032	0.0019*
6.15E+08	2.50E+05	2.80294	0.0051*
5.00E+06	2.50E+05	2.46616	0.0137*
6.15E+08	3.00E+08	2.20541	0.0274*
3.00E+08	2.50E+05	0.59987	0.5486
7.50E+07	5.00E+06	0.04728	0.9623
6.15E+08	5.00E+06	-0.41638	0.6771
6.15E+08	7.50E+07	-0.41960	0.6748
3.00E+08	5.00E+06	-2.01798	0.0436*
3.00E+08	7.50E+07	-2.60775	0.0091*

* significant difference at a probability level of $P \leq 0.05$.

Declaration*

I,

Name, First name

Born on

Matriculation number ,

hereby declare on my honor that the attached declaration,

- ☐ Homework/Presentation
- ☐ Bachelor Thesis
- ☐ Master Thesis
- ☐ Diplom Thesis,

has been independently prepared, solely with the support of the listed literature references, and that no information has been presented that has not been officially acknowledged.

Supervisor
Lecturer

Thesis topic

Semester

I declare, here within, that I have transferred the final digital text document (in the format doc, docx, odt, pdf, or rtf) to my mentoring supervisor and that the content and wording is entirely my own work. I am aware that the digital version of my document can and/or will be checked for plagiarism with the help of an analyses software program.

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