Phytosanitary Cold Treatment Against Anastrepha grandis (Diptera: Tephritidae)

Authors: Hallman, Guy J., Maset, Bruno Aguiar, Martínez, Elena I. Cancio, Cáceres Barrios, Carlos E., Vreysen, Marc J. B., et al.

Phytosanitary cold treatment against *Anastrepha grandis* (Diptera: Tephritidae)

Guy J. Hallman[1,*], Bruno Aguiar Maset[1,2], Elena I. Cancio Martínez[1], Carlos E. Cáceres Barrios[1], Marc J. B. Vreysen[1], Scott W. Myers[3], and Viwat Wornoayporn[1,4]

Abstract

Anastrepha grandis (Macquart) (Diptera: Tephritidae) is endemic to the lowland Andean region of South America and considered a quarantine pest of cucurbits by most tropical and subtropical countries outside of the infested region. Despite its regulatory significance, the only phytosanitary treatment available is a generic phytosanitary irradiation dose of 150 Gy that is accepted for all Tephritidae but that was developed without any data on radiotolerance of *A. grandis*. The objectives of this research were to determine the most cold-tolerant developmental stage of *A. grandis* and to estimate a time period required for a phytosanitary cold treatment in zucchini squash, *Cucurbita pepo* L. (Cucurbitaceae). The most cold-tolerant developmental stage of *A. grandis* in fruit was the 3rd instar, and the time required for a phytosanitary cold treatment in zucchini squash when treated at a minimum of 1.0 °C was estimated at ~23 d. This indicates that *A. grandis* is approximately as cold tolerant as the most cold-tolerant *Anastrepha* species known, i.e., *Anastrepha ludens* (Loew). However, in small-scale testing, no survivors were found at 14 d (*n* = 340) and the estimated time of 23 d needs to be confirmed by large-scale testing before it should be used commercially. As statistical estimates of extreme values (>99.9%) are not very reliable, and data from 3rd instars did not fit the model well, large-scale testing should be initiated at a treatment time <23 d to not result in an excessive commercial treatment dose.

Key Words: quarantine treatment; phytosanitation; South American cucurbit fly; *Cucurbita pepo*

Resumo

A espécie *Anastrepha grandis* (Macquart) (Diptera: Tephritidae) é endêmica na planície Andina, região da América do Sul, e considerada uma praga quarentenária de abóboras pela maior parte do mundo tropical e subtropical que não faz parte da região infestada. Apesar de ter importância regulamentada, o único tratamento fitossanitário disponível é uma dose genérica de irradiação de 150 Gy que foi aplicada contra qualquer Tephritidae, sem que houvesse quaisquer dados de radiotolerância dos insetos. Esta pesquisa determina o estágio larval de *A. grandis*, encontrado no interior dos frutos, mais tolerante ao frio (3º instar), e estima o tempo necessário (~23 dias) para o tratamento fitossanitário a frio em abobrinhas, *Cucurbita pepo* L. (Cucurbitaceae) quando submetidas a um mínimo de 1,0 °C. Isso indica que a *A. grandis* é aproximadamente tão tolerante ao tratamento de frio quanto a espécie de *Anastrepha* mais tolerante a esse mesmo tratamento já conhecida, a *A. ludens* (Loew). No entanto, em testes em pequena escala, não foram encontrados sobreviventes para 14 dias de tratamento (*n* = 340) e a dose estimada de 23 dias precisa ser confirmada por testes em larga escala antes que se permita a comercialização dos frutos. Como as estimativas de valores extremos (>99,9%) através de regressão não são muito confiáveis, e dados de 3º ínstares não se encaixaram bem de nenhuma maneira no modelo, testes em larga escala devem ser iniciados em uma dose consideravelmente menor que 23 dias para não resultar em uma dose excessiva de tratamento comercial.

Palavras Chaves: tratamento de quarentena; fitossanidade; mosca sul-americana das cucurbitáceas; *Cucurbita pepo*

Anastrepha grandis (Macquart) (Diptera: Tephritidae), one of the larger tephritid fruit flies, occurs in much of the low elevation Andean region of South America attacking fruits of the Cucurbitaceae (Silva & Malavasi 1993; Bolzan et al. 2015). It is considered a regulated pest of cucurbits by most tropical and subtropical countries where it does not occur. Despite its regulatory significance, no phytosanitary treatments have been developed against this pest. Although it is covered by the 150 Gy generic phytosanitary irradiation dose approved for all Tephritidae, no irradiation research has been published on *A. grandis* (Hallman 2012).

Placement in temperatures near 0 °C for 2 to 5 wk has been used for a century to disinfest fruits of tephritid fruit flies (Gould 1994; Heather & Hallman 2008). From then until the present, it has been the most commonly applied phytosanitary treatment for various types of citrus fruits exported from several countries, such as Argentina, Australia, Morocco, Spain, and the USA. It is also used for a variety of

---

other fruits such as *Physalis peruviana* L. (Solanaceae) exported from Colombia to the USA.

To verify the efficacy of a phytosanitary treatment, the most tolerant life stage present in the commodity should be determined, and then large-scale confirmatory testing is usually conducted with that stage to demonstrate that it is efficacious to a high degree of certainty (Heather & Hallman 2008). The objectives of this research were to determine the most cold-tolerant developmental stage of *A. grandis* and to estimate a time period required for a phytosanitary cold treatment in zucchini squash, *Cucurbita pepo* L. (Cucurbitaceae). *Cucurbita pepo* was the best of several hosts of *A. grandis* as measured by infestation index, fecundity, and fertility (Bolzan et al. 2015).

Materials and Methods

Specimens of *A. grandis* were obtained from fruits originating from South America in 2012 and reared in the Insect Pest Control Laboratory of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in Seibersdorf, Austria, on zucchini squash obtained from a local wholesaler in Vienna. Zucchini weighing 200 to 400 g were placed in cages (44 × 44 × 44 cm) with adult *A. grandis* for 6 to 8 h for infestation to occur and then held at 26 °C for 0 to 20 d to obtain insects of different stages. To ensure that insects in all sizes and ages within a stage were represented in the analyses, infested fruits were treated at all ages between 0 and 20 d after infestation. The ages for each stage were determined by dissection of infested fruits and established as follows: 0 to 3 d: egg; 4 to 7 d: 1st instar; 8 to 13 d: 2nd instar; and 14 to 20 d: 3rd instar. These time periods do not precisely coincide with their respective stages, as there is overlap at the days adjoining consecutive stages, but for the purposes of robust determination of the most tolerant stage, this scheme is useful. At ~20 d, late 3rd instars are present in the fruit, but adults will soon begin to emerge.

When the desired stage developed in the zucchini, the infested fruit samples were placed in plastic boxes (44 × 33 × 12 cm) in a 2 m³ cold treatment chamber (model SE-2000-4, Thermotron Industries, Holland, Michigan) at a target temperature of 1.1 °C for 8 to 14 d. Temperatures in the chamber and within the fruit were recorded (datalogger model OM-CP-OCTTEMP2000, Omega, Stamford, Connecticut) with Type T, 30-gauge thermocouples that were calibrated to 0.0 °C in a slurry of reverse osmosis–purified ice and water. Upon removal, the fruits were returned to 26 °C and held until *A. grandis* would have reached the late 3rd instar, which was calculated by subtracting the number of days between exposure to adults and placement in the cold treatment chamber from 21; an additional day was added to allow surviving insects to recover from the cold treatment before examination. This additional day simulates the minimum time required for treated fruit to reach an importing inspection point. Controls for all stages were also held at 26 °C until late 3rd instars developed.

Non-treated but infested fruits (controls) were held at 26 °C; the proportion of fruits held as controls depended on the developmental stage of the insect. Dead eggs, 1st instars, and early 2nd instars could not readily be observed upon visual examination of the fruits. Therefore, the non-treated "controls" in this case were not used to estimate the level of response (mortality) not attributable to the cold treatment. Instead, non-treated fruits were used to estimate the number of insects present in the fruit, i.e., the number treated. Thus, there was no estimate of natural (non-cold treatment induced) mortality for eggs and early instars. A large proportion (30 to 50 %) of infested fruits was not treated to minimize variation in the estimate of number of insects present in the fruits per replicate. As late 2nd instars and 3rd instars could readily be observed upon visual examination of the fruits, the

control for these stages was only needed to determine natural mortality, and ~10% of the fruits were held as controls. It is assumed that natural (non-cold treatment induced) mortality was similar in all stages tested.

Because the research depended upon infestation via oviposition to avoid making untested assumptions related to more artificial techniques used in phytosanitary treatment research (Hallman 2014), sometimes not all fruits were infested or they rotted before they could be used. Therefore, the number of replicates varied from 2 to 4 per stage–time combination.

After a total of 21 d at 26 °C pre and post cold treatment, the zucchinis were opened and examined for dead and live larvae. Larvae that were the color of live ones but not moving were kept until they were obviously dead or alive. Larvae that moved upon inspection or pupariated were considered survivors regardless of subsequent fate.

Data were subjected to probit analysis (PoloPlus, Petaluma, California). For each stage, only the lowest dose that resulted in 100% mortality and that was not followed by a higher dose resulting in 1 or more survivors was used in the analysis; i.e., doses ≥11 d for eggs and 1st and 2nd instars all resulted in 100% mortality, thus, only data from 8 to 11 d were used in the analyses for those 3 stages.

Results

The infestation rate of non-treated zucchini fruits varied over the course of the study from 0 to 68 recovered larvae per fruit; more heavily infested fruits as well as some other fruits were disintegrating and not used in the research. The mean ± SE cold-treatment temperature during the study was 1.04 ± 0.02 °C, and the inside of fruits reached that temperature nearly 1 d after placement in the cold treatment chamber.

Mean natural mortality in the controls of 3rd instars was 0.78 ± 0.39%. Although there was no measurement of natural mortality in non-treated earlier instars or eggs, it is assumed that natural mortal-

Table 1. Total numbers of *Anastrepha grandis* eggs and instars in zucchini squash, *Cucurbita pepo,* fruits treated at 1.1 ± 0.1 °C for 8 to 14 d.

Stage	Treatment time (d)[a]	Numbers of insects treated	Mortality (%)
Egg	8	526	76.0
	9	294	98.3
	10	328	99.1
	11	376	100
1st instar	8	246	63.0
	9	115	80.0
	10	176	96.6
	11	457	100
2nd instar	8	140	54.3
	9	148	88.5
	10	122	96.7
	11	1581	100
3rd instar	9	176	87.5
	10	74	93.2
	11	476	95.0
	12	112	100
	13	204	97.1
	14	340	100

[a]At 12 to 14 d for egg and 1st and 2nd instar = 100% mortality; 8 d for 3rd instar not done.

Table 2. Results of probit analysis of *Anastrepha grandis* eggs and instars in zucchini squash, *Cucurbita pepo*, fruits treated at 1.1 ± 0.1 °C for 8 to 14 d: χ^2, heterogeneity, and lethal dose (LD) estimates (d) for 90%, 99%, and ≈99.9968% ("probit 9") mortality (95% fiducial limits).

Stage	χ^2	Heterogeneity	LD90	LD99	LD≈99.9968
Egg	8.1	2.7	8.5 (8.2–9.0)	9.5 (9.0–11.5)	11.4 (10.1–17.2)
1st instar	5.6	2.8	9.3 (8.7–10.7)	10.6 (9.7–14.1)	12.7 (11.0–19.8)
2nd instar	5.1	2.5	9.1 (8.6–9.9)	10.1 (9.4–12.3)	12.0 (10.6–17.9)
3rd instar	10.4	2.6	9.5 (7.0–10.5)	13.4 (11.9–23.6)	23.2 (16.7–144)

ity in these stages was also low and would not significantly affect the results of the probit analysis.

Mortality of the stages of *A. grandis* was similar for eggs and 1st and 2nd instars, for which 100% mortality was achieved at 11 d (Table 1). Complete mortality of 3rd instars required 14 d. Estimates of doses to achieve various levels of mortality by probit analysis, log10 of dose, are given in Table 2. At 23.2 d, the estimate for 99.9968% (probit 9) mortality of 3rd instars was approximately twice the estimate for the other stages.

Discussion

Eggs and 1st and 2nd instars were more susceptible to cold treatment than 3rd instars. There was no estimate of natural (non-cold treatment induced) mortality in the eggs and early instars, and it was assumed to be negligible because natural mortality in the controls of late instars was negligible (mean = 0.78%). As natural mortality increases, efficacy of the treatment decreases as some of the mortality that would normally be ascribed to the treatment is in reality due to other causes. However, at the high levels of efficacy achieved with the doses used in this study, corrections for natural mortality would be negligible even if they were in the order of ~20%.

Cold tolerance in *A. grandis* follows that for most other tephritids (Hallman et al. 2013a,b) in that the 3rd instar was the most tolerant. The estimate to provide a very high level (99.9968%) of mortality of 3rd instars in zucchini fruit is 23.2 d. However, fit of the data to the probit model was not good, as exemplified by the upper 95% fiducial limit being 144 d. Because estimates of very high levels of efficacy are not always accurate (West & Hallman 2013), large-scale confirmatory testing, where at least 10,000 3rd instars are treated with no survivors, would usually be required to substantiate that the treatment was efficacious with a high degree of confidence.

Acknowledgments

References Cited

Bolzan A, Nava DE, Garcia FRM, Valgas RA, Smaniotto G. 2015. Biology of *Anastrepha grandis* (Diptera: Tephritidae) in different cucurbits. Journal of Economic Entomology 108: 1034–1039.

Gould WP. 1994. Cold storage, pp. 119–132 *In* Sharp JL, Hallman GJ [eds.], Quarantine Treatments for Pests of Food Plants. Westview Press, Boulder, Colorado.

Hallman GJ. 2012. Generic phytosanitary irradiation treatments. Radiation Physics and Chemistry 81: 861–866.

Hallman GJ. 2014. Insect thermotolerance comparing host infestation methods: *Anastrepha ludens* (Diptera: Tephritidae) reared in grapefruit or diet. Journal of Economic Entomology 107: 1377–1384.

Hallman GJ, Myers SW, Taret G, Fontenot EA, Vreysen MJB. 2013a. Phytosanitary cold treatments for oranges infested with *Bactrocera zonata* (Diptera: Tephritidae). Journal of Economic Entomology 106: 2336–2340.

Hallman GJ, Myers SW, El-Wakkad MF, Tadrous MD, Jessup AJ. 2013b. Development of phytosanitary cold treatments for oranges infested with *Bactrocera invadens* and *Bactrocera zonata* (Diptera: Tephritidae) by comparison with existing cold treatment schedules for *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology 106: 1608–1612.

Heather NW, Hallman GJ. 2008. Pest Management and Phytosanitary Trade Barriers. CAB International, Wallingford, United Kingdom.

Silva JG, Malavasi A. 1993. The status of honeydew melon as a host of *Anastrepha grandis* (Diptera: Tephritidae). Florida Entomologist 76: 516–519.

West M, Hallman GJ. 2013. Estimation of dose requirements for extreme levels of efficacy. Annual Conference on Applied Statistics in Agriculture, http://newprairiepress.org/agstatconference/2013/proceedings/10 (last accessed 23 May 2016).