Effects of Cold Treatment on Mortality of *Dysaphis cynarae* (Hemiptera: Aphididae) and *Copitarsia decolora* (Lepidoptera: Noctuidae) on Fresh Artichokes.

F. Urra y J. Apablaza[1]
Facultad de Agronomía e Ingeniería Forestal
Pontificia Universidad Católica de Chile
Casilla 306-22, Santiago, Chile

Abstract

Urra, F. and J. Apablaza. 2005. Effects of cold treatment on mortality of *Dysaphis cynarae* (Hemiptera: Aphididae) and *Copitarsia decolora* (Lepidoptera: Noctuidae) on fresh artichokes. *Dysaphis cynarae* and *Copitarsia decolora* are key pests of artichokes with quarantine status. Their mortality when stored near 0 ºC, for different lengths of time, was evaluated here. Experiments for *D. cynarae* were carried out in Pomaire and for *C. decolora* in Santiago (Región Metropolitana). Naturally aphid-infested artichokes were kept at 0.2 ºC. Live and dead aphids were periodically counted up to 28 days. For *C. decolora*, artichokes were artificially infested with one larva per unit and kept at 0.1 ºC. Dead larvae were periodically counted up to 21 days. The same procedure was used in a separate study at 18.0 ºC. There were no significant differences in aphid mortality between control and cold storage up to 14 days. After 21 and 28 days, mortality reached 53,0% and 64,3%, respectively, being significantly higher than that found on cold-free artichokes, but not enough for total aphid control. All *C. decolora* were alive after 3 days in cold storage. Mortality of fourth-instar larvae was significant after 14 days in cold storage. For second-instar larvae, mortality was significantly higher from 7 days on. Mortality was over 80% after 21 days in cold storage, and this may be useful for local market. At 18 ºC, mortality was very low and not significant, corroborating that cold storage may reduce infestations. The mortality that cold storage inflicted in both species is not enough for fresh export produce going to countries where these insects have quarantine status.

Key words: *Dysaphis cynarae*, *Copitarsia decolora*, fresh artichokes, cold treatment.
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INTRODUCTION

Artichoke, *Cynara scolymus* L. (Asteraceae), is native to the Mediterranean basin. It may be consumed fresh, preserved or frozen (Maroto, 1982; Giaconi y Escaff, 1995). About 3.000 ha are cultivated each year in Chile, mainly in the IV, V and Metropolitan Regions, where most of the production is sold at the local market (ODEPA, 2001). Most exports of fresh artichoke has gone to the USA; however, there has been a significant decrease in the exported volume (ODEPA, 2003).

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[1] Corresponding author: juapabl@uc.cl

The importance of insect pests in this crop varies with weather, harvest time, and final market. Among the insect species of quarantine importance in the USA are the artichoke sphid, *Dysaphis cynarae* (Theobald) and the vegetable caterpillar, *Copitarsia decolora* (Guenée).

Fuentes-Contreras *et al*. (1997), based on Stroyan (1985), stated that *D. cynarae* corresponds to the subspecies *D. lappae cynarae* (Theobald), while Blackman and Eastop (1984), Prado (1991), Artigas (1994), refer to the insect as *D. cynarae*, name that will be used in this study. *Copitarsia decolora* until recently was known as *Copitarsia turbata* (Herrich-Schaeffer). However, Angulo (2004) as well as Simmons and Pogue (2004) informed that this name is synonymous to *C. decolora*.

Since the 1996-1997 season, the USDA considered that the presence of aphid nymphs in fresh artichokes was cause of rejection (Asociación de Exportadores de Chile, A.G., Inspection Program of SAG/USDA/ASOEXPORT, Reports of the seasons 1995/96 and 1996/97). This induced the exporting companies to stop shipments to that country in order to prevent future implications. Soon after, D. *cynarae* was identified and considered as one of the reasons for the reduction in exports to that country. During prior seasons, *C. decolora* (Lepidoptera: Noctuidae) had been the main cause of rejection (Machuca and Arretz, 1988). Regarding chemical control , treatment with insecticides only are permitted if the products to be used are accepted by the purchasing country, and any non authorized residue motivates rejection of the lot. However, fumigation with methyl bromide causes serious damage to artichokes (Carvajal *et al*., 1991).

Physical methods such as cold and heat have been used successfully as disinfectant treatment for several insect pests, among them Tortricid moths (Neven and Rehfield, 1995; Chervin *et al*., 1997; Hansen, 2002), Mediterranean fruit fly (Tugwell, 1992; Armstrong *et al*., 1995; Jang *et al*., 2001) and some beetles (Delate *et al*., 1994; Dohino *et al*., 1999). Using cold to control quarantine insects on artichokes is possible, since the optimal post harvest conditions of the product include the use of temperatures close to 0°C and a relative humidity of 90%, for a period that fluctuates, depending on the variety, between 15 to 30 days (Ryall and Lipton, 1978; André *et al.*, 1980). Temperatures close to the freezing point tend to be lethal for insects that are not hibernating (Chiang, 1985). The objective of this study was to determine the effect of temperatures close to 0 ºC and exposure time duration on the mortality of *D. cynarae* and *C. decolora*, and to estimate the potential use of this technique as quarantine treatment for artichokes.

MATERIALS AND METHODS

The Argentinean artichokes used in this study came from a commercial crop located in Pomaire, Metropolitan Region, Chile.

Effect of low temperatures on Dysaphis cynarae

This study was carried out in cold chambers with an approximate capacity of 800 m^3, during October and November 2002. It consisted of an experiment with six treatments: 0, 3, 7, 14, 21 and 28 days of storage at 0.2 ºC (ranging from 0 to 1 ºC). The chamber was set at 0 ºC but the temperaure was also measured with a thermometer registering maximum and minimum values per day. This procedure was repeated four times during the study.

Artichokes naturally infested with aphids, were picked from the field, then taken to the cold chambers, and randomly distributed in groups of five units within open polyethylene bags. These constituted the experimental unit. On day 0, live and dead aphids were counted in a group of five randomly chosen artichokes. The rest of such groups were kept in storage in the cold chamber. After 3 days other bag with five infested artichokes was removed, and kept for 24 h at 18.0 °C, in order to reactivate live insects. Then, the number of live or dead aphids per bag was registered. Likewise, the same procedure was repeated after 7, 14, 21 and 28 days, successively, until completing four replicates.

The aphids were counted with a Nikon stereoscopic magnifying glass of 30X. If the insect under observation remained immobile, it was touched with a fine brush to determine if it was dead or alive.

Effect of low temperature on Copitarsia decolora larvae

For this study a cold chamber of approximately 60 m^3, from the Pontificia Universidad Católica de Chile, School of Agronomy and Forestry Engineering was used from December 2002 until February 2003.

In the first preliminary study, fourth-instar larvae were reared in alfalfa, under laboratory conditions, in Santiago. For the other experiments, second-instar larvae were employed, all of them originating from a single female hatched in the laboratory from an egg. The artichokes were placed in 0.5 to 1.0 L, wide-mouth glass containers, and covered with fine cloth. Each artichoke was artificially infested with one *C. turbata* larva. The experimental unit consisted of groups of six or nine randomly selected artichokes, kept at 20°C for 24 h prior to placement at the corresponding temperature. The purpose was to facilitate the larval infestation of the artichoke, thus simulating what would happen in the field.

The infested artichokes in the experiments at 0.1 °C, were introduced in the cold chamber and five or nine removed after 3 days. These were kept for 24 h at 18.0 °C, in order to re-activate live insects. Immediately after this, the number of live and dead larvae in the artichokes was recorded. This procedure was repeated the same way after 7, 14, and 21 days; until completing all four replicates. The chamber temperatures were cheked with a maximum and minimum thermometer.

Gradually the artichokes bracts were removed until finding the insects and recording their status. Occasionally it was necessary to use a Nikon (30X) stereoscopic magnifying glass. If the insect remained immobile, it was stung with a dissection needle to determine if it was dead or alive.

For the experiment at 18.0 °C, the process was similar, but using a Bioref VV-19, Puig & Larraguibel refrigerated incubator, programmed at this temperature.

Statistical analysis

In all the studies, the mortality percentage per experimental unit was calculated. Data were transformed to an arcsin $\sqrt{x}$ (Zar, 1999) in order to perform the analysis of variance, and separate the means with the Tukey test (Bancroft, 1968) whenever there was a significant difference at 5%. To compare larvae mortality in different stages of *C. Decolora*, the "t" test was used. Also, simple regression analyses were performed to describe the effect of exposure time on insect mortality. A SigmaStart 2.0, Jandel Corporation program was utilized for these cases.

Table 1. Mean mortality of *Dysaphis cynarae* on artichokes stored at 0ºC for various time periods.

Treatment (days)	Mean mortality (%)
0	13.10 ± 7.23 a[1]
3	15.62 ± 5.91 ab
7	37.35 ± 15.05 abc
14	28.83 ± 9.75 abc
21	53.05 ± 21.78 bc
28	64.30 ± 21.52 c

[1] Means followed by the same letter are not significantly different (ANOVA, Tukey-Kramer, P>0.05)

RESULTS AND DISCUSSION

Dysaphis cynarae mortality

Table 1 shows that aphid mortality at 0 and 3 days of cold storage, were the lowest. There were no significant differences between 3, 7 and 14 day treatments versus treatment 0. The highest mortalities were reached during day 21 and 28, being significantly different to controls (P=0.006). *D. cynarae* mortality at 0.2 ºC increased with exposure time (Figure 1; P<0.001). Yet, this mortality level would be insufficient to control *D. cynarae* during artichoke post harvest. If extrapolating from the linear regression analysis that was performed, 100% insect mortality would be achieved on day 49, and with a linear behavior during that period. This time period would exceed optimal storage conditions for artichokes that is only 15 to 30 days (Ryall and Lipton, 1978; André *et al*.,1980). Aphid predators such us adult and larval coccinellids, syrphid maggots and spiders were active in the field where artichokes were harvested for this study. However, aphid death due to those predators was ruled out since none of them was found during this research. The possibility of hymenopteran parasitoids was also disregarded, because no microwasps or parasitized aphids were observed during the inspection of artichokes.

Copitarsia decolora larvae mortality

Results demonstrated that mortality of fourth-instar larvae at 7 days was not significantly different to that of control, but cold at 14 and 21 days significantly reduced the number of live larvae (Table 2, P=0.001).

Second-instar larvae had higher mortality from day 7 onwards (Table 2, P=0.05). However, there was no significant difference between treatments 7, 14, and 21 days.

Although mortality of second-instar larvae appeared higher than that of fourth-instar larvae, no significant difference was found when compared each treatment between both instars ("t" test: P=1.00, 0.15, 0.49, 0.72 for 3, 7, 14 and 21 days, respectively). In this study, smaller larvae were not significantly more susceptible to cold than large ones. These results would indicate that cold treatment could be useful in reducing the number of live larvae and therefore, the damage of the product allotted to the local market, but insufficient for foreign markets where this insect has quarantine importance.

All larvae raised at 18.0 °C, exhibited low mortality, reaching a maximum of only 5.6% at 21 days, and without significant differences along time (P = 0.09). Therefore, mortality of second-instar larvae was due to low temperature.

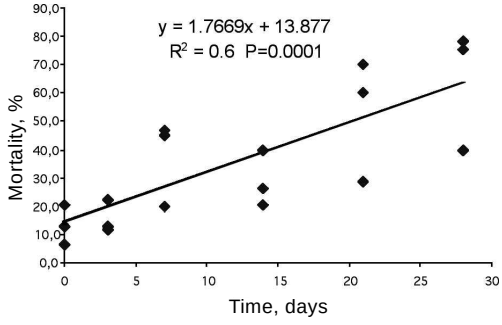

Figure 1. Mortality of *Dysaphis cynarae* on artichokes stored at a mean temperature of 0.2º C for various time periods.

Table 2. Mortality (%) of *Copitarsia turbata* 2nd and 4th instar larvae at 0.1º and 18.0 ºC for various time periods

Treatment (days)	Temperature		
	0.1 ºC		18.0 ºC
	Fourth instar	Second instar	Second instar
3	0.0 ± 0.0 a[1]	0.0 ± 0.0 a[1]	0.0 ± 0.0 a
7	27.8 ± 40.4 a b	69.5 ± 26.3 b	0.0 ± 0.0 a
14	61.1 ± 34.4 b	77.8 ± 31.4 b	2.8 ± 5.6 a
21	72.2 ± 27.2 b	80.5 ± 26.3 b	5.6 ± 11.1 a

[1] *Means followed by the same letter in a column are not significantly different (ANOVA, Tukey-Kramer, P>0.05)*

A recommendation, which stems from the observations of this study, would be to keep the artichokes for 7 days at 0 ºC to achieve 70% small *Copitarsia decolora* larvae control. This would be comparable to Hansen's recommendation (2002) to control *Cydia pomonella* (Lepidoptera: Tortricidae) in cherries keeping them at 3.3 ºC during 7 days; apparently implying that *C. pomonella* is more sensitive to cold than *C. decolora*. Likewise Rahemi and Zare (2002) recommend for *Plodia interpunctella* (Lepidoptera: Pyralidae) control in dry figs, 2 ºC for 25 h, indicting that this moth also is more susceptible to cold than *C. decolora.* Recommendations of Dohino *et al.* (1999) to control eggs of various insects in stored products, indicate temperatures of -18 ºC, demonstrating the high resistance to cold that this developmental stage has as compared to larvae.

CONCLUSIONS

Low temperatures have an important effect on *Dysaphis cynarae* and *Copitarsia decolora* mortality, but the treatment studied is not enough for the total control required in markets where these insects quarantine. Given that artichokes noticeably deteriorate with temperatures under 0 ºC and that this temperature has a duration that varies between 15 and 30 days, depending on the variety and post harvest handling, among other factors, cold in itself is not a satisfactory treatment for these two insect pests.

RESUMEN

Dysaphis cynarae y *Copitarsia decolora* son plagas importantes en alcachofa y cuarentenarias para Estados Unidos. En esta investigación se determinó la mortalidad que causan temperaturas cercanas a 0 ºC en estos insectos. El estudio con *D. cynarae* se realizó en Pomaire, Región Metropolitana, y el de *C. decolora* en la Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Para *D. cynarae* se utilizaron alcachofas naturalmente infestadas, que se mantuvieron a 0,2 °C. Los pulgones vivos y muertos se registraron periódicamente hasta los 28 días. Para *C. decolora* cada alcachofa se infestó con una larva y se mantuvieron a 0,1 ºC. Las larvas muertas se registraron periódicamente hasta los 21 días. Otro grupo de larvas se trató de manera similar, pero a 18,0 ºC. No hubo mortalidad de áfidos significativamente diferente al tratamiento 0 días en frío, después de 3, 7 y 14 días en frío. A los 21 y 28 días, la mortalidad alcanzó 53,0% y 64,3%, respectivamente, siendo significativamente mayores a la del testigo, pero insuficiente para control total de *D. cynarae* en postcosecha. No hubo mortalidad de *C.*

decolora tras 3 días en frío. A partir de los 14 días hubo mortalidad significativa en las larvas de cuarto subestado. Para las larvas de segundo subestado, la mortalidad fue significativamente superior a partir de los 7 días. La mortalidad superó al 80% después de 21 días, lo cual sería útil para el mercado interno, pero insuficiente en situaciones donde esta plaga es cuarentenaria. A 18,0 ºC, la mortalidad fue baja y constante en el tiempo, corroborando que la mortalidad anterior fue causada por frío.

Palabras clave: *Alcachofas, Copitarsia decolora, Dysaphis cynarae,* frío.

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