

Life cycle, phenology and economic importance of the walnut husk fly *Rhagoletis completa* Cresson (Diptera: Tephritidae) in northern Italy

CARLO DUSO & GIULIO DAL LAGO

Department of Environmental Agronomy and Crop Science, University of Padova, Agripolis, Viale dell'Università 16 - 35020 Legnaro, Padova, Italy

Abstract. The walnut husk fly *Rhagoletis completa* Cresson, a pest originating from North-America, was detected for the first time in Europe in 1991. The life cycle and phenology of *R. completa* were studied, during two successive seasons, in two commercial orchards located in northern Italy. The pest develops one generation per year. Fly emergence lasted from early July to the second half of August. Oviposition was detected from late July to early September, with peaks between August 5 and 18. First instar larvae were recorded from early August and mature larvae left husks from late August onwards to pupate in the soil. The seasonal patterns of nuts infestation showed rapid growths in August following oviposition peaks. Nuts infestation levels in the untreated plots of the two orchards, ranged from 74-91% in the first year to 89-91% in the following season. Relatively dark shell surfaces were linked to a weight reduction in nuts and kernels and to an increase in darkened and mouldy kernels. Observations made in an untreated orchard for additional 3 years confirmed these trends. Baited Pherocon AM were effective in monitoring flies but catches on woody green spheres were better correlated with the first relevant oviposition phases. When pesticides were sprayed with the correct timing, i.e. against eggs or first instar larvae, infestation was kept to acceptable levels with a single application. Considering average yields, nuts prices and costs for insecticide use, 1-2 insecticide treatments are economically viable. Other Implications are discussed.

Résumé. Cycle de vie, phénologie et importance économique de la mouche des brous du noyer *Rhagoletis completa* Cresson (Diptera : Tephritidae) au nord de l'Italie. La mouche des brous du noyer *Rhagoletis completa* Cresson est un ravageur originaire d'Amérique du Nord. Elle a été détectée pour la première fois en Europe en 1991. Son cycle de vie et sa phénologie ont été étudiés durant deux saisons successives dans deux vergers commerciaux du nord de l'Italie. Le ravageur développe une seule génération par an. La période d'émergence dure depuis le début juillet jusqu'à la seconde moitié d'août. L'oviposition a été détectée de fin-juillet à début-septembre, avec un pic entre le 5 et le 18 août. Les larves de premier stade ont été observées à partir de début-août tandis que les larves matures ont quitté les écales à partir de la fin-août pour puper dans le sol. La séquence saisonnière d'infestation des noix a montré une croissance rapide en août à la suite des pics d'oviposition. Les niveaux d'infestation des noix dans les parcelles non traitées des deux vergers ont été de 74-91% la première année et de 89-91% la seconde. La surface tachée relative des écales a été mise en relation avec la réduction en poids des noix et des coques et à une augmentation de la fréquence des coques moisies et noircies. Des observations faites dans un verger non traité durant 3 années supplémentaires ont confirmé ces tendances. Le « *Pherocon AM* » appâté a été efficace pour la surveillance des mouches mais la collecte sur les sphères vertes a été surtout corrélée avec la première phase notable d'oviposition. Lorsque les pesticides ont été répandus en temps utile, entre autre contre les oeufs et les larves de premier stade, l'infestation a été gardée à des niveaux acceptables avec une application unique. Prenant en compte les rendements moyens, les prix des noix et les coûts des traitements insecticides, une ou deux applications sont rentables. D'autres implications sont discutées.

Keywords: *Rhagoletis completa*, Diptera Tephritidae, Persian walnut, life cycle, phenology, economic importance, pest control.

The walnut husk fly *Rhagoletis completa* Cresson (Diptera: Tephritidae) is a key pest of the Persian walnut in the U.S.A. (Boyce 1934; Olson & Buchner, 2002). In 1991, the occurrence of *R. completa* was reported for the first time in Europe, in particular

in southern Switzerland (Merz 1991). This author identified some specimens collected in the late 1980s and preserved in museum collections. There were no records of *R. completa* in European walnut orchards until the pest was recorded in northeastern Italy (Duso 1991). In the 1990s, *R. completa* was recorded in various regions of northern and central Italy (Ciampolini & Trematerra 1992; Duso 1994; Trematerra *et al.* 1995), in Switzerland (Mani *et al.* 1994), and in Slovenia

E-mail: carlo.duso@unipd.it

Accepté le 2 mars 2006

(Seljak 1999). Recently, the pest has been detected in Croatia and in localities close to the borders of Austria and Hungary (Seljak, *in lit.*).

The biology and behaviour of *R. completa* have been studied in detail in southern California (Boyce 1931, 1934). Later, additional studies were carried out in California and other states of the USA (Michelbacher & Ortega 1958; Gibson & Kearby 1978; Riedl & Hoying 1980; Kasana & AliNiazee 1996). In the U.S.A., the pest has one generation per year. Diapausing pupae overwinter in the first layers of the soil and adults emerging in the subsequent summer feed on honeydew, yeasts and leaf exudates (Kasana & AliNiazee 1995a). Mating occurs 6-8 days after emergence followed by oviposition 1-2 weeks later. The oviposition period lasts over a month (Boyce 1934; Riedl & Hoying 1980; Kasana & AliNiazee 1996). Visual and, to a lesser extent, olfactory stimuli affect searching for oviposition sites (Cirio 1972). Usually, a single batch of eggs is laid on the fruit in a cavity created by the ovipositor. Total fecundity can reach a maximum of 400 eggs per female (Boyce 1934). Egg hatch and larval development depend on temperature (Kasana & AliNiazee 1995b). In field conditions, 5-10 days are often required for egg hatch and 3-5 weeks for larval development. Larvae feed on walnut husk tissues and, once mature, leave the infested fruits to fall to the soil where they pupate (Boyce 1934; Jones 1967).

Larval feeding affects husk tissues, causing shell staining and darkened kernels. High infestations cause premature nut fall and additional harvesting costs (Boyce 1934). Since the role of natural enemies in affecting *R. completa* populations seems to be negligible, chemical control is often necessary (Boyce 1934; Nickel & Wong 1966; Riedl *et al.* 1989). Adults can be caught by a number of traps, but yellow rectangular sticky traps baited with ammonium carbonate and green spheres simulating nuts proved to be more effective (Riedl & Hislop 1985; Riedl *et al.* 1989). Sampling of population in nuts is recommended after the first adult catches in order to detect the beginning of oviposition, which is the crucial phase for controlling the pest (Riedl & Hoying 1980).

The occurrence of *R. completa* in northern Italy was initially associated with significant economic damage in some orchards. Since classical biological control appeared to be unrealistic in the short term, it was necessary to develop control measures based on phenological data and effective monitoring techniques. Sampling was carried out in a number of orchards to look at inter-site and temporal variation. Finally, the economic damage caused by *R. completa* was estimated in order to optimise pesticide use.

Materials and methods

Orchard features

Most of the experiments were carried out in two orchards (their acronyms are MS and CN) located in the lowlands of Treviso (Veneto region, north-eastern Italy) during 1992 and 1993.

The MS orchard was rectangular in shape and measured approximately 0.8 hectares. Some old plants belonging to local ecotypes of *Juglans regia* L. were contiguous to the orchard. The plants of the cultivar Franquette were 15 years old, 10-12 m high, not pruned and the planting pattern was 8 x 9 m. Soil was medium-textured and grass cover was constantly maintained. Flush irrigation was sometimes provided.

The CN orchard was also rectangular in shape and measured approximately 14 hectares. The plant characteristics were similar to those reported for the MS orchard, but the plants were less vigorous probably because of different soil features. Soil texture was very rich in gravel and grass cover was constantly maintained. Drop irrigation was provided. Untreated plants of *J. regia* were contiguous to the experimental area. In both orchards diseases were controlled with the use of copper fungicides. Insecticides were seldom applied to control *Cydia pomonella* (L.).

Additional observations on the phenology of the pest were performed in a small orchard (LA) of approximately 1000 m² constituted by plants belonging to local ecotypes and not treated with pesticides. In this orchard, close to the commercial orchards, the pest was monitored from 1994 to 1996.

Observations on the life cycle and the phenology of *Rhagoletis completa*

Adult emergence was monitored in 1992 and 1993 by using cages, rectangular in shape (1.5 x 0.5 m), made of aluminium and covered with tulle. The cages were placed near the trunk. A yellow sticky trap was inserted into each cage to make it easier to count emerged flies. The latter were collected and examined in the laboratory to assess sex-ratio.

Adult flight in the orchards was monitored in 1992 and 1993 by using traps of different shape, material and colour. They included: yellow rectangular sticky traps in PVC (14 x 23 cm), effective in capturing a number of dipterans (e.g. Duso & Vettorazzo 1996); Pherocon AM (Trécé) already used in the U.S.A. to monitor *R. completa* populations (Riedl & Hoying 1980); the same traps baited with 3 g of ammonium carbonate (Riedl & Hoying 1981); woody green spheres (diameter 6.8 cm) similar to those used by Riedl & Hislop (1985) in California. The green colour was obtained according to the reflectance curve of Franquette nuts by mixing various pigments. The spheres were painted using this colour and then glued together. All these traps were placed on the foliage, in particular on outer shoots, at approximately 2 m height.

The traps were sampled weekly, the flies were counted and then removed. The yellow sticky traps were cleaned and covered in spray glue every week. These traps and Pherocon AM traps were renewed every 3 weeks. Ammonium carbonate was added every week. The green spheres were also cleaned every week and covered in a new layer of glue.

In the MS and CN orchards, 5 cages, 3 yellow sticky traps, 3 Pherocon AM, 3 Pherocon AM baited with ammonium carbonate and 10 green spheres were placed in untreated plots. In the untreated orchard (LA), 3 baited Pherocon AM were used for monitoring the pest during 1994-1996.

In the commercial orchards (MS, CN) sampling of nuts was performed on 10 untreated plants (10 nuts per plant), every week from June to October of 1992 and 1993, in order to assess population densities of *R. completa*. The same sampling procedure was applied in LA orchard in 1994 and 1995. During 1996, sampling involved 22 plants (2 nuts per plant). Nuts infested by *R. completa* were dissected to evaluate the density of different developmental stages (eggs, first, second and third larval instars). Nuts drop was also evaluated on the same plants.

In September 1992, 500 *R. completa* pupae collected in the MS orchard were placed inside 100 test-tubes (5 pupae per test-tube) containing sand and plugged with cotton. The material was then placed outside and examined every week from May to October during the years 1993, 1994 and 1995 in order to evaluate the proportion of emerging adults.

The effect of insecticides on *Rhagoletis completa* populations

During 1992, MS and CN orchards were divided into plots treated or not treated with insecticides to control *R. completa*. In the MS orchard, dimethoate (1500 g of active ingredient per hectare) was used on 8 August 1992. During the vegetative season, sampling was carried out on 10 plants selected from plots treated with dimethoate and on 10 plants selected from plots not treated with insecticides. Each treatment comprised two replicates of 5 plants. The same type and number of traps mentioned above were placed in the dimethoate plots to monitor flies.

In the CN orchard, insecticide treatments (fenitrothion, 1500 g of active ingredient per hectare) were applied to control *C. pomonella* on May 26 and July 14 of 1992. Later, dimethoate and fenitrothion (both 1500 g of active ingredient per hectare) were applied in different plots (8 August) to control *R. completa*. Sampling was carried out on: 10 plants selected from dimethoate plots, 10 plants selected from fenitrothion plots, and 10 plants selected from untreated plots. Each treatment included 2 replicates of 5 plants. The same type and number of traps mentioned for the control were placed in the dimethoate plots.

Damage assessment

The total nut production of 5 plants per treatment was examined at harvest time in both orchards. The number of not infested (NI) and infested nuts was calculated for each plant. Symptoms were divided into 4 classes based on the extent of darkened shell surface: L (low, less than 1/3), M (moderate, between 1/3 and 2/3), H (high, more than 2/3), VH (very high, completely dark).

The relationship between the extent of darkened shell surface and the economic damage is not precise. Therefore, 16 samples belonging to the different classes were weighed; each sample contained 25 nuts for a total of 400 nuts per class. Then, the kernels of each sample were divided into 4 additional classes based on symptoms (no symptoms, slightly darkened, severely darkened, darkened and mouldy) and weighed. This evaluation was made during 1992 and 1993.

Statistical analysis

Differences in *R. completa* infestation levels among the control and insecticide treated plots were analysed using the

REPEATED option of Proc GLM of SAS (SAS Institute Inc. 1989) with a one-way analysis of variance considering the date as a repeated measure. The means were separated by using the option REGWQ of SAS and the level of significance was $P = 0.05$. For presentation of the results, the discussion was focused on analysis of contrasts (Zar 1984). A logarithmic transformation, i.e. $\log(y + 1)$, was applied to the data before carrying out an ANOVA.

The ANOVA was used to evaluate differences among treatments concerning yield parameters. Means were separated by using Duncan's test.

The effectiveness of traps in capturing flies and differences in catch numbers between the control and insecticide treated plots were evaluated by using Proc FREQ of SAS (SAS Institute 1999) applying Friedman two-way analysis of variance by ranks (Siegel & Castellan 1988) and performing multiple comparison procedures as described by Ipe (1987).

Differences in the amount of infested nuts between the control and insecticide treated plots were evaluated by using Proc NPAR1WAY of SAS (SAS Institute 1999) applying the Kruskal-Wallis one way analysis of variance by ranks (Siegel & Castellan 1988).

Results

Phenology and infestation levels in MS orchard

In 1992, adult emergence in the control plots lasted from July 15 to August 18 (fig. 1A). Sex-ratio was close to 1:1 with a slight predominance of females. Oviposition was detected from July 28 to August 18 (fig. 1B). Usually, a single puncture was found per nut and the average number of eggs per puncture was 21.8. First instar larvae were observed on August 5 and third instar larvae from August 11 onwards. Mature larvae left the husks from late August in order to pupate in the soil. The amount of infested nuts increased from August onwards reaching maximum levels in September when a severe nuts drop was recorded (figs 2A, 2B).

Observations on the diapause, made in the laboratory, showed that most adults (84.2%) emerged in the season following pupation (1993) and the remaining (8.8%) in the subsequent year (1994). In a number of cases (7%) adults did not emerge but parasitoids were not included among mortality factors.

In 1993, fly emergence lasted from July 7 to August 11 (fig. 3A) and oviposition from July 29 to August 25 (fig. 3B). First instar larvae were recorded on August 4 and third instar larvae from August 11 onwards. Mature larvae were observed leaving the husks in the second half on August. Infestation levels gradually increased reaching the highest values in mid-September when nuts drop was also significant (figs 4A, 4B).

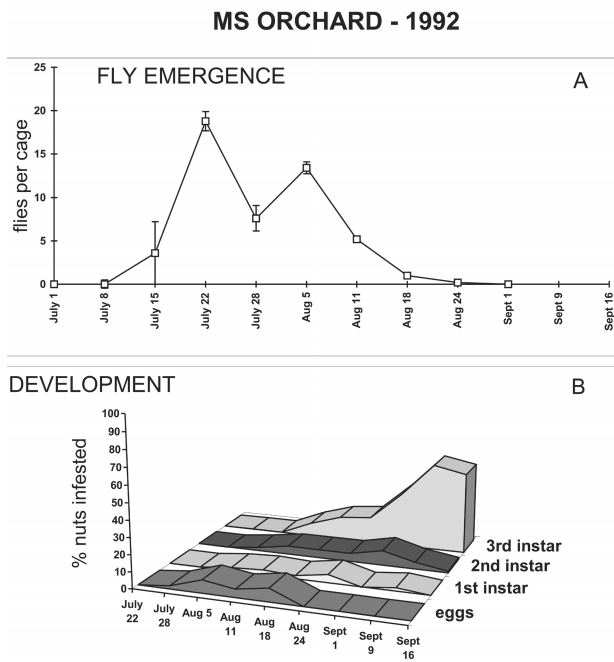


Figure 1
Adult emergence (A) and life cycle (B) of *R. completa* in the control plots of MS orchard (1992).

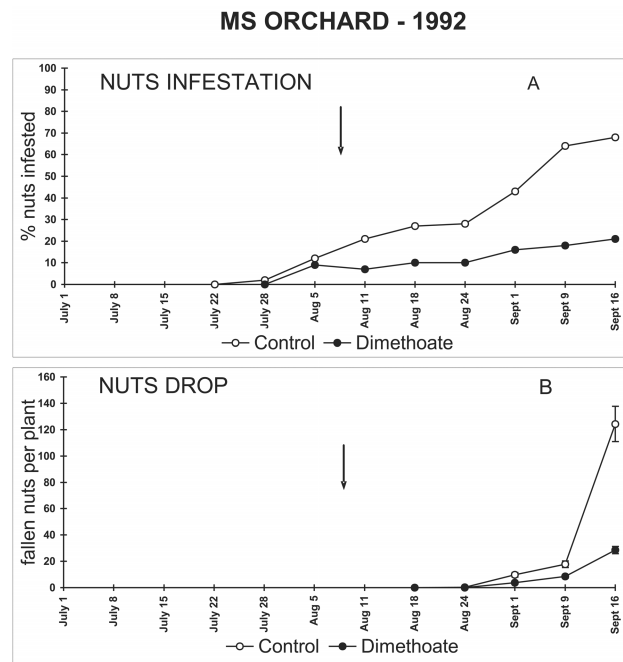


Figure 2
Infestation trends (A) and nuts drop (B) recorded in the control and in dimethoate plots of MS orchard (1992). The arrow indicates the date of dimethoate spray (August 8).

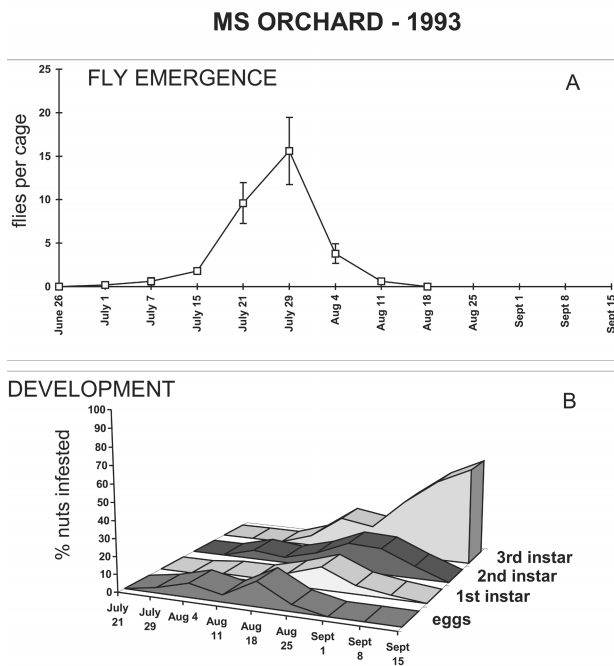


Figure 3
Adult emergence (A) and life cycle (B) of *Rhagoletis completa* in the control plots of MS orchard (1993).

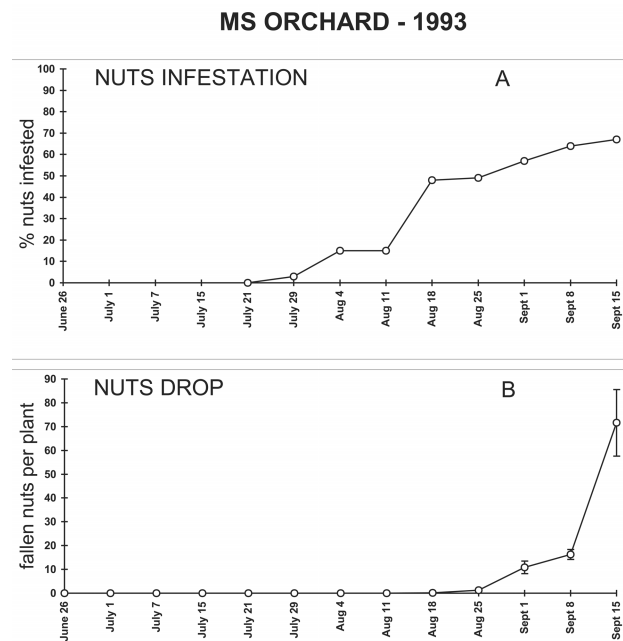


Figure 4
Infestation trend (A) and nuts drop (B) recorded in the control of MS orchard (1993).

Phenology and infestation levels in CN orchard

In the CN orchard, during 1992, flies emerged from July 15 to August 18 with a peak on July 28. In the control, oviposition was recorded from late July to the beginning of September with maximum values on August 11. First instar larvae were observed on August 5, third instar larvae two weeks later. Infestation levels reached 45% in September, before harvesting. Nuts drop dramatically increased in September (60.9 nuts per plant on September 16).

In 1993, fly emergence lasted from July 7 to August 11 reaching relatively high levels between July 29 and August 4. Oviposition on untreated nuts was detected from early to late August with a peak on August 18. However, first instar larvae were recorded on August 4, suggesting that the beginning of oviposition was not detected effectively. Third instar larvae occurred from August 18 onwards and nuts left by them were recorded from late August onwards. Infestation trends showed an exponential growth in August reaching maximum levels (74% of infested nuts) in mid September. Nuts drop was consistent with this trend (62.4 nuts per plant on September 15).

The effectiveness of different traps in the MS orchard

During 1992, the amount of catches depended on

trap used ($F_r = 145.05$; $P < 0.0001$). The effectiveness of yellow sticky traps was lower than that of Pherocon AM traps ($P < 0.0001$). Unbaited Pherocon AM were less effective than Pherocon AM traps baited with ammonium carbonate ($P < 0.0001$). The first flies were detected on July 15 on all these traps.

On untreated plants, the number of adults caught on baited Pherocon AM (flies per trap per week) appeared to be similar to that of woody green spheres from July to mid August but later green spheres seemed more attractive (fig. 5A). Since the surface of Pherocon AM is more or less twice that of green spheres (145 cm² vs. 322 cm²) the higher performance of the latter is emphasized.

The trends of catches on green spheres or on baited Pherocon AM were compared with oviposition phases (fig. 5B). Pherocon AM appeared to be more effective than green spheres before oviposition while green spheres gave better results from the beginning of reproduction. The two peaks of catches on the green spheres corresponded to the more relevant oviposition phases. Regarding baited Pherocon AM, this coincidence was observed only for the first peak.

During 1993, the effect of trap confirmed to be significant ($F_r = 137.66$; $P < 0.0001$). Baited Pherocon AM were more attractive than commercial Pherocon AM ($P = 0.008$) and yellow sticky traps ($P < 0.0001$).

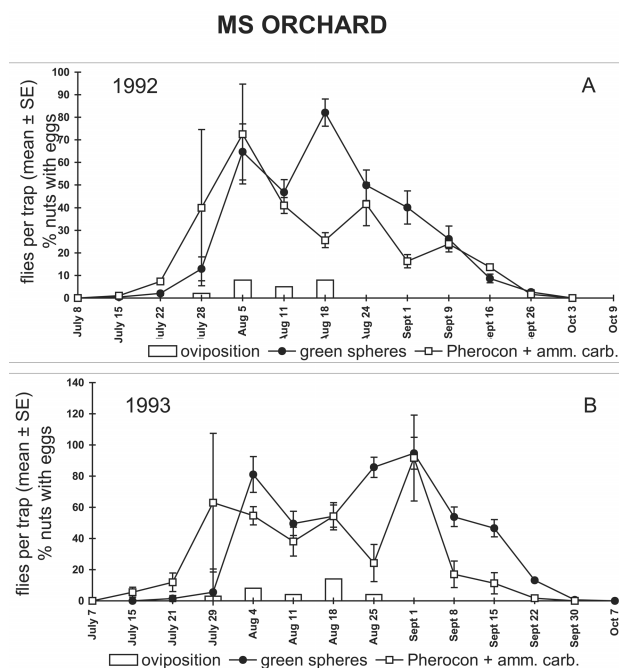


Figure 5
Fly catches on different traps, and oviposition phases recorded in the control of MS orchard in 1992 (A) and 1993 (B).

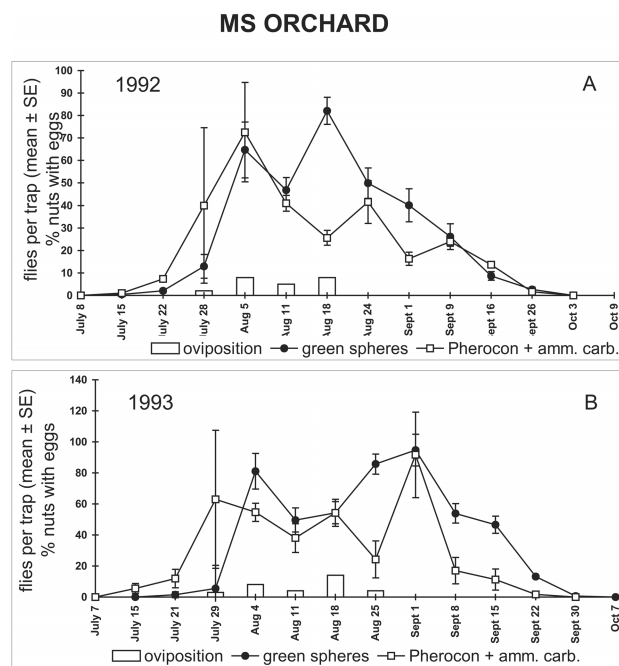


Figure 6
Fly catches on different traps, and oviposition phases recorded in the control of CN in 1992 (A) and 1993 (B).

Pherocon AM were more effective than yellow sticky traps ($P = 0.022$).

According to the results of 1992, baited Pherocon AM attracted more flies than green spheres before the first ovipositions (fig. 5B). The first increase in catches on green spheres was associated to a relevant oviposition phase (fig. 5B). A second peak of catches, recorded in the second half did not anticipate a significant oviposition.

The effectiveness of different traps in monitoring *Rhagoletis completa* in the CN orchard

In 1992, the effectiveness in capturing flies was affected by the trap used ($F_r = 42.28$; $P < 0.0001$). Baited Pherocon AM were more effective than commercial Pherocon AM ($P < 0.0001$) and yellow sticky traps ($P < 0.0001$). Differences among the latter were also significant ($P < 0.0001$).

Fly catches reached relatively high levels corresponding with oviposition (fig. 6B). Baited Pherocon AM were more attractive at the beginning of oviposition and green spheres in the first half of August. Later, catch trends were similar.

In 1993, the amount of catches was affected by trap type ($F_r = 45.05$; $P < 0.0001$). Baited Pherocon AM captured more flies than unbaited Pherocon AM and yellow sticky traps ($P < 0.0001$) and differences among the latter were confirmed ($P = 0.012$).

Trends of catches on baited Pherocon AM and green spheres in 1993 were similar to those recorded in the previous season and in the MS orchard. Green spheres were more useful than baited Pherocon AM in predicting the onset of oviposition (fig. 6B).

The effect of insecticides on *Rhagoletis completa* populations

Regarding MS orchard, catch values in the cages placed in the dimethoate plots were lower than those

detected in the control ($F_r = 4.95$; $P = 0.03$). However, in the period preceding the application of dimethoate the catches on traps were comparable in the control and in dimethoate plots ($F_r = 4.66$; $P = 0.138$ for yellow traps; $F_r = 1.38$; $P = 0.239$ for Pherocon AM; $F_r = 3.73$; $P = 0.053$ for Pherocon AM baited with ammonium carbonate; $F_r = 0.002$; $P = 0.96$ for green traps).

The use of dimethoate caused a decline in catch numbers in yellow traps ($F_r = 16.28$; $P < 0.0001$), Pherocon AM ($F_r = 15.60$; $P < 0.0001$) and green spheres ($F_r = 137.56$; $P < 0.0001$). In contrast, there were no differences in catches between the treatments by using baited Pherocon AM ($F_r = 0.53$; $P = 0.464$).

Infestation levels in the control and in dimethoate plots were comparable before insecticide application (August 8) ($F = 0.95$; d.f. = 1, 18; $P = 0.301$). Later, infestation and nuts drop increased more in the control than in dimethoate plots ($F = 29.8$; d.f. = 1, 18; $P < 0.0001$; $F = 74.2$; d.f. = 1, 18; $P < 0.0001$, respectively; figs 2A, 2B).

In CN orchard, the amount of catches on traps in the period preceding the application of dimethoate were comparable in the control and in dimethoate plots ($F_r = 1.43$; $P = 0.232$ for yellow traps; $F_r = 0.112$; $P = 0.738$ for Pherocon AM; $F_r = 1.96$; $P = 0.161$ for Pherocon AM baited with ammonium carbonate; $F_r = 0.768$; $P = 0.38$ for green spheres). The use of dimethoate was associated to a decline in catch numbers on Pherocon AM ($F_r = 8.69$; $P = 0.003$) and green spheres ($F_r = 72.62$; $P < 0.0001$). There were no differences between the treatments by using yellow traps ($F_r = 1.24$; $P = 0.265$) and baited Pherocon AM ($F_r = 0.812$; $P = 0.367$).

Infestation levels in the control and in the treated plots were comparable in the date preceding insecticide application ($F = 0.31$; d.f. = 2, 26; $P = 0.735$) but the latter reduced infestation size ($F = 71.45$; d.f. =

Table 1. Incidence of different damage classes recorded at harvest time of 1992 in treated or untreated plots of MS and CN orchards.

Farm	Treatment	Damage class (darkened shell)				
		NI (0)	L (<1/3)	M (1/3-2/3)	H (>2/3)	VH (3/3)
		% (N)	% (N)	% (N)	% (N)	% (N)
MS	Control	8.98 (449)	10.12 (506)	15.06 (753)	15.54 (777)	50.30 (2515)
	Dimethoate	56.88 (2844)	14.88 (744)	12.56 (628)	8.82 (441)	6.86 (343)
CN	Control	26.30 (1315)	3.98 (199)	12.96 (648)	15.48 (774)	41.28 (2064)
	Dimethoate	69.22 (3461)	15.90 (795)	4.70 (235)	4.24 (212)	5.94 (297)
	Fenitrothion	67.26 (3363)	12.86 (643)	6.90 (345)	5.86 (293)	7.12 (356)

2, 26; $P < 0.0001$). The analysis of contrasts showed that dimethoate had a significant effect on the pest populations ($F = 116.44$; d.f. = 1, 26; $P < 0.0001$). Similar results were obtained with fenitrothion ($F = 97.03$; d.f. = 1, 26; $P < 0.0001$). The effectiveness of the two insecticides was comparable ($F = 0.88$; d.f. = 1, 26; $P = 0.356$).

Nuts drop was significantly reduced in treated plots ($F = 65.52$; d.f. = 2, 26; $P < 0.0001$; fig. 5B). Both insecticides had a significant effect on the nuts drop ($F = 103.36$; d.f. = 1, 26; $P < 0.0001$; $F = 83.09$; d.f. = 1, 26; $P < 0.0001$; for dimethoate and fenitrothion respectively). The results obtained using the two compounds were similar ($F = 1.10$; d.f. = 1, 26; $P = 0.303$).

Damage assessment

In the MS orchard, at harvest time of 1992, the total number of infested nuts was significantly higher ($Z = 2.61$; $P = 0.009$) in the control (91%) than in dimethoate plots (43.1%). The proportion of nuts belonging to different classes (L, M, H, VH) is reported in tab. 1. The most important differences were observed for class VH (shell completely darkened) for which values reached 50.3% in the control and 6.9% in dimethoate plots.

The amount of infested nuts, in CN orchard during 1992, was significantly higher ($H = 9.42$; $P = 0.009$) in the control (73.7%) than in treated plots (30.8% for dimethoate, 32.7% for fenitrothion). Multiple comparison among treatments showed a significant effect of pesticide application: dimethoate and fenitrothion reduced the amount of infested nuts with respect to the control ($P = 0.017$ and $P = 0.032$, respectively). The incidence of different damage classes is reported in tab. 1.

In the evaluation made in 1992, the weight of nuts belonging to class VH was significantly lower ($F = 27.2$; d.f. = 4, 75; $P < 0.0001$; tab. 2) than that of class NI but also of nuts belonging to classes L, M

and H. Similar results were obtained regarding kernel weight ($F = 21.2$; d.f. = 4, 75; $P < 0.0001$; tab. 2). The percent of kernels without symptoms was not different between NI and L classes but it significantly decreased in classes M, H and VH ($F = 180.8$; d.f. = 4, 75; $P < 0.0001$; tab. 2). A similar trend was observed regarding kernels partially darkened ($F = 59.8$; d.f. = 4, 75; $P < 0.0001$; tab. 2). The amount of severely damaged kernels (darkened or darkened and mouldy) was not different among NI, L and M classes while it increased in H and VH classes ($F = 26.3$; d.f. = 4, 75; $P < 0.0001$; $F = 44.9$; d.f. = 4, 75; $P < 0.0001$; tab. 2). Nuts belonging to class L can be excluded from those economically damaged. Therefore, in MS orchard infestation levels were reduced reaching 80.9% in the control and 28.2% in treated plots. Regarding CN orchard, net infestation values reached 69.7% in the control, 14.9% in dimethoate plots, 19.9% in fenitrothion plots.

During 1993, the amount of infested nuts in untreated plots of the MS orchard reached 89.4%. The incidence of different damage classes was similar to that of the previous year (9.4% for class L, 14.4% for class M, 15.8% for class H, and 49.9% for class VH). In untreated plots of CN orchard, the amount of infested nuts during 1993 reached 90.6%. The incidence of highly infested nuts increased more than in the previous season: 5.4% of infested nuts belonged to class L, 15.6% to class M, 20% to class H, and 49.5% to class VH.

Damage assessment calculated for the 1993 confirmed most of the results shown in 1992. The weight of nuts belonging to classes H and VH was significantly reduced, as was the weight of kernels belonging to classes M, H and VH (tab. 3). The percent of kernels without symptoms was not different between NI and L classes while this value significantly decreased in kernels of classes M, H and VH (tab. 3). The amount of kernels partially darkened, completely darkened, or darkened and mouldy was significantly

Table 2. Weight of nuts, weight of kernels and percentage of kernels with symptoms recorded on different damage classes (1992).

Data followed by different letters indicate significant differences at Duncan's test ($P < 0.01$).

Damage class (darkened shell)	Nuts weight (g)	Kernels weight (g)	Kernels without symptoms (%)	Kernels partially darkened (%)	Kernels completely darkened (%)	Kernels darkened and mouldy (%)
NI (0)	236.87 a	92.81 a	96.75 a	0 c	0.25 b	3 c
L (< 1/3)	231.87 a	92.5 a	94.5 a	1.75 c	2.5 b	1.25 c
M (1/3-2/3)	232.81 a	99.68 a	82.75 b	12 b	2.75 b	2.5 c
H (> 2/3)	239.68 a	96.56 a	60.25 c	19.75 a	11 a	9 b
VH (3/3)	210 b	75 b	42.25 d	22.75 a	14 a	21 a

Table 3. Weight of nuts, weight of kernels and percentage of kernels with symptoms recorded on different damage classes (1993). Data followed by different letters indicate significant differences at Duncan's test ($P < 0.01$).

Damage class (darkened shell)	Nuts weight (g)	Kernel weight (g)	Kernels without symptoms (%)	Kernels partially darkened (%)	Kernels completely darkened (%)	Kernels darkened and mouldy (%)
NI (0)	247.18 a	91.87 a	98 a	0 c	0 c	2 c
L (< 1/3)	248.43 a	95.62 a	92.5 a	1.75 c	2 c	3.75 c
M (1/3-2/3)	244.68 a	80 b	70.5 b	20 b	2.75 c	6.75 bc
H (> 2/3)	228.75 b	74.37 bc	53.25 c	27.25 a	8.5 b	9.75 b
VH (3/3)	214.37 c	65.62 c	32 d	28.5 a	22.2 a	18.5 a

higher in classes H and VH than in classes NI and L; values regarding class M were close to those of classes NI and L or intermediate (tab. 3).

Observations conducted in orchard LA

Additional observations, performed in an untreated walnut orchard (LA) from 1994 to 1996, showed that fly catches, detected by baited Pherocon AM, peaked on August 19, August 24 and August 15, respectively in 1994, 1995 and 1996. Oviposition peaks were observed on August 12 (1994), August 18 (1995) and August 15 (1996). Infestation levels reached maximum values of 46% in August 1994, 65% in September 1995, and 36% in August 1996. During these growing seasons a high number of nuts was infested by *C. pomonella*. These nuts were rarely infested by *R. completa*. Moreover, in 1994 and 1996, a proportion of infested nuts fell before ripening because of drought.

Discussion

The most important phenological phases of *R. completa* observed during 1992 and 1993, were similar to those recorded in Oregon, U.S.A. (Kasana & AliNiazee 1996). The latter study was performed in orchards containing the Franquette variety. In southern California, *R. completa* emergence appeared to be delayed and lasted longer than that observed in northern Italy, thus affecting the subsequent phenological phases (Boyce 1934). In northern California and Missouri the phenology of the pest also showed differences with respect to our findings (Riedl & Hoying 1980, Gibson & Kearby 1978).

Oviposition by *R. completa* is related to host plant conditions and in particular to husk hardness, nut growth and possibly other factors (Boyce 1934; Riedl & Hoying 1980; Kasana & AliNiazee 1996). From our data, it can be seen that the oviposition period was slightly different between the two seasons within the same orchard while there were some differences among orchards. However, oviposition peaks were recorded

in the same period (August 5-18). This coincidence was also observed in other two orchards containing the Franquette variety and in untreated orchards (local ecotypes) located in the same area (unpublished data of the authors). In orchard LA, where observations continued for 3 subsequent years, oviposition peaks were synchronised over the seasons despite the very different climatic conditions. Trials conducted during 1998 and 1999, in another untreated orchard, showed a substantial coincidence between the peaks of fly catches and those of oviposition (S. Beria *et al. in lit.*). Walnut varieties affect ovipositional preferences and times (Riedl & Hoying 1980; Kasana & AliNiazee 1995b). The Franquette and *J. regia* local ecotypes considered in the present work could have similar characteristics with respect to ovipositional behaviour. In southern California oviposition peaks were detected in the same week for five subsequent seasons despite differences in emergence patterns (Boyce 1934).

Climate, and in particular temperature, affects husk fly emergence, adult behaviour and larval development (Kasana & AliNiazee 1994, 1996). The similarity found in the present study, regarding oviposition peaks and larval development duration between 1992 and 1993, could also be explained by temperature records. In both years, the mean temperature during the first three weeks of August ranged from 21 to 27°C but values were close to 25-26°C on several days. In the laboratory, reproductive behaviour and larval development were affected positively by temperatures ranging from 24 to 28°C (Kasana & AliNiazee 1994). Observations performed in orchards located in areas close to the Alps, where summer temperatures reach lower values than in the area of this study showed that oviposition and larval development were clearly delayed suggesting the importance of temperature in affecting phenological phases (S. Marchio *et al. in lit.*).

The number of punctures per nut and of eggs per puncture were very similar to those reported by Kasana & AliNiazee (1995b) for the same variety.

The seasonal patterns of nuts infestation showed rapid growths in August especially following oviposition peaks. Nuts infestation levels in the control plots of the two orchards, assessed before harvesting, ranged from 45 to 68% in 1992 and from 67 to 74% in 1993. Final damage levels were higher than those reported (74-91% in 1992, 89-91% in 1993) because harvest occurred 1-2 weeks after the last sampling and fallen nuts were included. These values are similar to those reported for the U.S.A. (Riedl & Hoying 1980; Kasana & AliNiazee 1996) and show that *R. completa* has become a key pest in northern Italy. The spread of the pest in Europe has been slower than expected: France seems to be free from *R. completa* occurrence despite the species has been common in Italy and in Switzerland for a decade. In contrast, the colonization of Slovenia, Croatia (and probably other Countries) by *R. completa* from North-eastern Italy has been faster. The presence of unsprayed walnuts in agroecosystems of these regions has probably favoured this phenomenon.

The economic damage recorded in north-eastern Italy warrants appropriate control measures. When pesticides were sprayed with the correct timing, infestation was kept to acceptable levels by a single application. Pesticide timing is linked to effective monitoring techniques. The effectiveness of baited Pherocon AM, confirms previous reports (Riedl & Hoying 1981; Riedl & Hislop 1985). Green spheres gave more precise indications for using pesticides against larval populations, as reported by Riedl *et al.* (1989), but nuts sampling seems to be necessary for predicting the onset and the extent of oviposition.

In both years, nuts not infested or belonging to class L showed no differences concerning certain yield parameters (weight of nuts, weight of kernels, symptoms on kernels). The weight of nuts belonging to classes H and VH was significantly reduced during 1993. In the same year the weight of kernels belonging to classes M, H and VH was also reduced. During 1992, these effects were recognized for class VH only. Differences among the seasons may be due to the dry conditions that occurred during summer 1993. The amount of kernels with symptoms increased from class M to class H. Completely darkened nuts showed 32-42% of kernels without symptoms but their commercial value was questionable. Regarding *R. completa*, an economic threshold evaluation may be made by considering as infested nuts only those belonging to classes M, H and VH.

A single insecticide application saved 52.7% of the production in MS orchard and 49.8-54.8% in CN orchard. Considering average productions, nuts

prices and costs for insecticide use, a single treatment is economically viable. The contiguity of treated and untreated plots suggests that these percentages may increase in the practice. Observations carried out in CN orchard, from 1993 to 1995, showed that two insecticide treatments applied in early August and mid-August reduced infestation levels to 5-8% at harvest. Presently, problems caused by *C. pomonella* and *R. completa* in Northern Italy induce nuts growers to apply at least 3-4 insecticide treatments in orchards. In our trials we did not observe spider mite increases after the insecticide applications. However, the problem of additional costs for controlling secondary pests should be considered carefully.

Acknowledgements. We would like to thank V. Girolami and S. Zangheri for their suggestions, G. Pavanello for his continuous support during field observations, and A. Pozzebon for performing a number of statistical analyses. Data coming from LA orchard were collected in cooperation with S. Beria.

References

- Boyce A.M. 1931. The diapause phenomenon in insects, with special reference to *Rhagoletis completa* Cresson. *Journal of Economic Entomology* **24**: 1018-1024.
- Boyce A.M. 1934. Bionomics of the walnut husk fly, *Rhagoletis completa*. *Hilgardia* **8**: 363-579.
- Ciampolini M., Trematerra P. 1992. Diffusa presenza di Mosca delle noci (*Rhagoletis completa* Cresson) nel Nord Italia. *Informatore agrario* **48**: 52-56.
- Cirio U. 1972. Osservazioni sul comportamento di ovideposizione della *Rhagoletis completa* Cresson (Diptera: Tephritidae) in laboratorio. *Atti IX Congresso Nazionale Italiano di Entomologia* **1**: 99-117.
- Duso C. 1991. Sulla comparsa in Italia di un Tefritide nearctico del nocce: *Rhagoletis completa* Cresson (Diptera: Tephritidae). *Bollettino di Zoologia Agraria e di Bachicoltura* **23**: 203-209.
- Duso C. 1994. Note sulla distribuzione ed il ciclo biologico di *Rhagoletis completa* Cresson (Diptera: Tephritidae) nell'Italia settentrionale. *Atti XVII Congresso Nazionale Italiano di Entomologia* **1**: 589-592.
- Duso C., Vettorazzo E. 1996. Osservazioni sul comportamento e sulla dannosità di *Bradysia paupera* Tuomikoski (Diptera: Sciaridae) in coltura protetta. *Bollettino di Zoologia Agraria e di Bachicoltura* **28**: 23-40.
- Gibson K.E., Kearby W.H. 1978. Seasonal life history of the walnut husk fly (*Rhagoletis completa*) and husk maggot (*Rhagoletis suavis*) in Missouri. *Environmental Entomology* **7**: 81-87.
- Ipe D. 1987. Performing the Friedman test and the associated multiple comparison test using PROC GLM. *Proceedings of the SAS Users Group International Conference* **12**: 1146-1148.
- Jones S.C. 1967. The walnut husk fly and its control. *Proceedings of the Nut Growers Society of Oregon and Washington* **53**: 59-62.
- Kasana A., AliNiazee M.T. 1994. Effect of constant temperature on development of the walnut husk fly, *Rhagoletis completa*. *Entomologia Experimentalis et Applicata* **73**: 247-254.
- Kasana A., AliNiazee M.T. 1995a. Adult flight dynamics of walnut husk fly (Diptera: Tephritidae) in the Willamette Valley of Oregon. *Pan-Pacific Entomology* **71**: 142-148.
- Kasana A., AliNiazee M.T. 1995b. Ovipositional preferences of the walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae) on various fruits, vegetables and varieties of walnuts. *Journal of Entomological Society of British Columbia* **92**: 3-7.

- Kasana A., AliNiazee M.T. 1996.** Seasonal phenology of the walnut husk fly, *Rhagoletis completa* Cresson (Diptera: Tephritidae). *Canadian Entomologist* **128**: 377-390.
- Mani E., Merz B., Brugnetti R., Schaub L., Jermini M., Schwaller F. 1994.** Zum Auftreten der beiden amerikanischen Fruchtfiegenarten *Rhagoletis completa* Cresson und *Rhagoletis indifferens* Curran in der Schweiz (Diptera: Tephritidae). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **67**: 177-182.
- Merz B. 1991.** *Rhagoletis completa* Cresson und *Rhagoletis indifferens* Curran zwei wirtschaftlich bedeutende nordamerikanische Fruchtfiegen, neu für Europa (Diptera: Tephritidae). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **64**: 55-57.
- Michelbacher A.E., Ortega J.C. 1958.** A technical study of insects and related pests attacking walnuts. *California Agriculture Experimental Station Bulletin* **764**: 62-69.
- Nickel J.L., Wong T.T.Y. 1966.** Control of the walnut husk fly, *Rhagoletis completa* Cresson, with systemic insecticides. *Journal of Economic Entomology* **59**: 1079-1082.
- Olson W.H., Buchner R.P. 2002.** Leading edge of plant protection for walnuts. *Horttechnology* **12**: 615-618.
- Riedl H., Hoying S.A. 1980.** Seasonal patterns of emergence, flight activity and oviposition of the walnut husk fly in Northern California. *Environmental Entomology* **9**: 567-571.
- Riedl H., Hoying S.A. 1981.** Evaluation of trap designs and attractants for monitoring the walnut husk fly, *Rhagoletis completa* Cresson (Diptera: Tephritidae). *Zeitschrift für angewandte Entomologie* **91**: 510-520.
- Riedl H., Hislop S.A. 1985.** Visual attraction of the walnut husk fly (Diptera: Tephritidae) to colored rectangles and spheres. *Environmental Entomology* **14**: 810-814.
- Riedl H., Barnett W.W., Coates W.W., Coviello R., Joos J., Olson W.H. 1989.** Walnut husk fly (Diptera: Tephritidae): evaluation of traps for timing of control measures and for damage predictions. *Journal of Economic Entomology* **82**: 1191-1196.
- SAS Institute Inc. 1999.** *SAS/STAT User's Guide*, Version 8. SAS Institute Inc., Cary, NC.
- Seljak G. 1999.** Orehova muha (*Rhagoletis completa* Cresson) – nov nevaren škodljivec orehov v Sloveniji. *Revija za Sadjarstvo Vinogradništvo in Vinarstvo* **10**: 12-15.
- Siegel, S., Castellan, N.J. 1988.** *Nonparametric statistics for the behavioural science*. McGraw-Hill, Singapore, 309 p.
- Trematerra P., Paparatti B., Girgenti P. 1995.** Attenzione alla presenza della mosca delle noci. *Informatore agrario* **47**: 74-76.
- Zar J.H. 1984.** *Biostatistical analysis*. Prentice-Hall International Inc., Englewood Cliffs, 718 p.