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Nearctic *Chymomyza amoena* (LOEW) (Diptera: Drosophilidae) remains a domestic species in Switzerland

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Nearctic *Chymomyza amoena* females have been found to oviposit on or in parasitized unripe fallen apples in orchards, gardens and meadows in Cantons Zürich, Aargau and Ticino. Sites investigated were near houses on farms, in villages and near a former hotel in Zürich. Parasitized fruits in Europe represent an open ecological niche for *C. amoena*, which breeds in these fruits in the eastern United States. This niche also enables *C. amoena* to remain a domestic drosophilid in Switzerland.

Key words: *Chymomyza amoena*, Switzerland, domestic drosophilid, breeding sites, open ecological niche, parasitized apples

INTRODUCTION

Nearctic *Chymomyza amoena* (LOEW) in Switzerland has entered the forest drosophilid community (BURLA & BÄCHLI, 1992; BAND *et al.*, 1998). In both Cantons Zürich and Ticino, it has repeatedly been captured among the non-domestic mycophagous and *obscura* group *Drosophila* species. In Canton Ticino it has been bred from acorns *Quercus robur*, chestnuts *Castanea sativa*, hazelnuts *Corylus avellana*, wild sweet cherries *Prunus avium* and wild apples *Malus sylvestris* collected in wooded areas in the Maggia Valley, and from acorns in the Bolle di Magadino, a forest preserve close to Gordola (BURLA & BÄCHLI, 1991, 1992; BAND *et al.*, 1998). There was only one report of its emergence from domestic apples *Malus domestica* collected in a yard in 1991 (BURLA & BÄCHLI, 1992). Apples yielding *C. amoena* and other *Drosophila* species in September, 1990, were collected under trees near Someo and Bignasco (BURLA & BÄCHLI, 1991) and in July, 1997, were collected along a trail in a wooded area near Someo (BAND *et al.*, 1998).

Although this species has been found to breed in parasitized unripe and ripe fallen apples over wide area in the United States (BAND, 1988a, 1988b, 1995, 1996), fallen apples collected and inspected in both Cantons Zürich and the Maggia Valley, Canton Ticino in July, 1997, showed little evidence of parasitism. There was no evidence of tunneling by either codling moth *Cydia pomonella* larvae or *Rhynchites* spp. weevil larvae in the apples collected. *Rhynchites* weevil punctures in the apples had not been followed by weevil oviposition. Nor had *C. amoena* females used the punctures for oviposition in unripe damaged fallen apples collected at various sites in Canton Zürich in July, 1997 (BAND *et al.*, 1998). Nevertheless, females in replicate cultures of a Zürich stock, which was established at Michigan State University in August, 1997, and maintained on laboratory medium, ovi-

posed on parasitized unripe green Michigan apples when supplied with them in July, 1998.

Chymomyza eggs are distinctive (THROCKMORTON, 1962; SCHUMANN, 1987) and *C. amoena* is the only *Chymomyza* species known to breed in parasitized fruits (apples, pears, plums) (BAND, 1988a, 1988b, 1995, 1996). BAND (1988b, 1989, 1994) used *C. amoena* egg deposition in apples and other fruits to show aggregated oviposition existed and that behavior among Michigan and Virginia *C. amoena* females was similar.

Here we report that females of Swiss *C. amoena* natural populations are using parasitized fallen domestic apples for oviposition in two cantons in northern Switzerland, Zürich and Aargau, and in southern Canton Ticino. Placement of eggs on parasitized apples appears significantly affected by the increased presence of codling moth *Cydia pomonella* in Canton Ticino. Yeasts were also isolated from the apples although *Chymomyza* are more typically associated with filamentous fungi (GRIMALDI, 1986; BAND *et al.*, 1998). Trees in unsprayed noncommercial orchards on farms, hillsides, yards or village squares were all near sites of human habitation, hence "domestic" locations (DOBZHANSKY, 1965), in both cantons. Consequently, *C. amoena* remains a domestic species although it has entered the forest habitat via nut and wild fruit breeding and continuing attraction to newly damaged trees.

MATERIALS AND METHODS

In general, parasitized or damaged domestic apples under trees in yards or unsprayed orchards were gathered, and inspected for the presence of *C. amoena* eggs and larvae with the aid of a dissecting microscope. The number and distribution of the eggs were noted: stem, calyx, hole. Parasitized apples were also cut open to determine if *C. amoena* eggs had been deposited within a tunnel made by a pest larva and the number of eggs within the hole counted. *C. amoena* eggs from apples with eggs were transferred to vials containing LAKOVAARA's malt medium. The vials were numbered, and emergence, if any, recorded. In many cases, eggs had already hatched but larvae had failed to survive.

Microbial samples were plated, as before (BAND *et al.*, 1998), but this time emphasis was on microbial content of apples with *C. amoena* eggs. Culture medium for the isolation of fungi was as follows: malt extract (Difco), 3% (v/v) with agar, 1.5% (v/v) is the basal medium. The initial enrichment medium contained 35 mg/l Rose Bengal (Sigma) and 10 ml/l of medium Penicillin G/Streptomycin sulfate (stock solution: 10000 U/ml Pen; 10mg/ml Strep). Both Rose Bengal and the antibiotics were added sterile to the medium at the time Petri dishes were poured.

Canton Zürich

The farm at Hönggerberg was visited on 14.7.98 and permission obtained to gather fallen apples in the orchard. A total of 37 were collected. On 15.7.98 28 small, mostly green parasitized apples were collected under the trees in the hillside orchard adjacent to the road to the Hotel Zürichberg. Both locations had been visited in July 1997 but the Hönggerberg orchard had had few fruits due to damage by cold weather while apples at the Hotel Zürichberg location had showed little evidence of parasitism and no evidence of *C. amoena* oviposition.

Fifty green, firm apples under trees at Otelfingen were also gathered on 15.7.98.

Canton Aargau

Two sites at Mägenwil were also visited on 15.7.98. Site I contained an apple tree in a yard; fruits were large and red as were fallen fruits; 36 fallen fruits were collected. Site II contained several apple trees; parasitized fallen fruits were mostly small, green, firm and the site more shaded; 49 fallen apples were collected.

Canton Ticino

Valle Blenio: Apples under trees in and around Acquarossa were collected and inspected between 17.7.98 and 18.7.98. Codling moth and lesser apple worm *Grapholita prunivora* had attacked the apples. The delta region where much farming activity occurs is too sunny for shade loving *C. amoena* and a considerable distance from any woods.

Chestnuts were gathered in a mixed forest below Corzoneso. An altitudinal transect for drosophilids present at Alteniga (860 m), below Corzoneso (680 m) and the wooded area below Acquarossa (530 m) was carried out to compare with transect findings from Cevio to Cerentino in the Maggia Valley in July 1997 (BAND *et al.*, 1998). Baits were set and collections made morning and evening 18–20.7.98. The woods at Alteniga were primarily alder *Alnus* sp. and beech *Fagus sylvatica*, below Corzoneso they were chestnut *Castanea sativa*, alder and beech and the wooded area below Acquarossa was dominated by locust *Robinia pseudoacacia* and beech.

Southern Ticino

Apples collected in a meadow at Castelrotto (400 m) on 21.7.98 were small, green and parasitized; 18 were gathered. Twenty-one plums on the ground nearby were also collected but damaged plums lacked evidence of parasitism since plum curculio *Conotrachelus nenuphar* is absent in Europe. Chestnuts in a forest above Purasca (380 m) were gathered 20.7.98 as were apples under 3 trees. Apples were of mixed sizes; 30 were gathered. Both Castelrotto and Purasca are in the Malcantone region.

At Castel San Pietro in the Valle di Muggio a large apple tree provided considerable shade for some of the fallen apples. Collecting was confined to the shaded region. Apples were of various sizes; most were green and had been parasitized. Dissected, one was found to contain a codling moth larva; another a weevil larva. A total of 50 fallen apples were gathered.

Acorns in July 1998 in Bolle di Magadino were mostly dry and few in number in contrast to July 1997 (BAND *et al.*, 1998). Two green fallen damaged and 14 brown parasitized acorns from the previous year were gathered.

RESULTS

The existence of widespread apple pest invasion in summer 1998 enabled demonstration that *C. amoena* females use parasitized unripe fallen apples for oviposition in July in both northern and southern Switzerland as has been found in the United States (BAND, 1988a, 1988b, 1994, 1995). Tab. 1 shows the total number of fruits or nuts gathered, the number not damaged, the number damaged but lacking eggs, and the number with *C. amoena* eggs at each place where *C. amoena* eggs were found. The percentage of fruits or nuts having eggs at each place is calculated by dividing the number with eggs by the total number of fruits or nuts collected. Tab. 2 considers the distribution of eggs on apples at each place: stem, calyx, hole.

Tab. 1. Existence of *C. amoena* eggs in parasitized fruits and nuts in July 1998. The % fruits/nuts with *C. amoena* eggs at each place is calculated by dividing the number of fruits/nuts with eggs in the sample by the total number of fruits/nuts collected at that place.

Place	Fruit/nut	Total	No. <i>C. amoena</i>		With eggs	No. Eggs	%
			No hole	with hole			
Hönggerberg	Apples	37	8	24	5	13	13.5
Hotel Zürichberg	Apples	28	1	23	4	7	14.3
Mägenwil I	Apples	36	1	34	1	2	2.8
Mägenwil II	Apples	49	5	38	6	32	12.2
Corzoneso	Chestnuts	73	20	52	1	3	0.1
Castelrotto	Apples	18	5	10	3	28 *	16.7
Castelrotto	Plums	21	3	17	1	1	4.8
Purasca	Chestnuts	111	12	98	1	4 **	0.1
Purasca	Apples	30	8	17	5	37	16.7
Castel San Pietro	Apples	50	14	15	21	154 *	42.0
Bolle di Magadino	Acorns	16	3	9	4	13 ***	25.0
Total apples (7 places)		248	42	161	45	273	18.7

* = 1 larva; ** = 4 larvae; *** = 3 pupae cases

Where holes with eggs occurred also at the stem or calyx, corrections were made to increase the number of eggs in such holes or tunnels as indicated by the numbers in parenthesis. Tab. 3 compares the distribution of eggs over apples in Cantons Zürich and Aargau and in southern Canton Ticino. Tab. 4 gives the number of *C. amoena* emerging at the various places. Tab. 5 shows the numbers of *Drosophila* species collected at various elevations above Acquarossa. However in the Valle Ble-nio *C. amoena* still seems confined to chestnut forests and woods.

As shown in Tab. 1 at most places where apples were found to contain *C. amoena* eggs, the number of eggs over all apples with eggs indicates clustering. In northern Switzerland average number of eggs per apple ranges between 2 (n=1) and 5.3 ± 2.2 (n=6), both at Mägenwil. In southern Ticino, the average increases to between 7.3 ± 1.3 (n=21) and 9.3 ± 6.8 (n=3). *C. amoena* was found in two places in Canton Ticino in 1988, one very near Castel San Pietro. Other differences appear in Tab. 2. Apples in the north appeared to have tunnels (and holes for exiting pest larvae) at the side and egg deposition occurred here. This is especially the case at

Tab. 2. Oviposition of *C. amoena* eggs on/in apples. If eggs were in a hole at the stem or calyx, the number in parenthesis indicates the remaining eggs that were not in the hole at the stem or calyx. The total number of eggs in holes, given in parenthesis, has been increased upwards by the numbers in holes at the stem or calyx.

Place	Stem	Oviposition on apples			Total
		Calyx	Hole		
Hönggerberg	0	2	11		13
Hotel Zürichberg	2	1 (0)	4 (5)		7
Mägenwil I	0	2 (0)	0 (2)		2
Mägenwil II	0	0	32		32
Castelrotto	0	19 (0)	9 (28)		28
Purasca	0	5 (0)	32 (37)		37
Castel San Pietro	7 (5)	57 (43)	90 (106)		154
Total	9 (7)	86 (45)	178 (221)		273

Tab. 3. Comparison of oviposition of *C. amoena* eggs on/in parasitized apples between places in the Zürich area and places in Southern Ticino.

Area	Stem	Calyx	Hole	Total
Northern areas	2	5 (2)	47 (50)	54
Southern Ticino	7 (5)	81 (43)	131 (171)	219
Total eggs	9 (7)	86 (45)	178 (221)	273

Chi-square comparisons for egg oviposition in the two regions

$\chi^2 = 15.53$, d.f. = 2, P = 0.0004 ($\chi^2 = 8.14$, d.f. = 2, P = 0.0171)

Tab. 4. Emergence of *C. amoena* from substrates collected in July 1998.

Collecting date	Locality	Substrate	Total No.	Males	Females
14 July	Hönggerberg	Apples	3	0	3
21 July	Castelrotto	Plums	1	1	0
21 July	Purasca	Chestnuts	4	2	2
22 July	Purasca	Apples	2	2	0
23 July	Castel San Pietro	Apples	15 *	6	8
24 July	Bolle di Magadino	Acorns	4	2	2
Total			29 *	13	15

* one escaped before sexing

Hönggerberg and Mägenwil II localities. In the three localities in southern Ticino more eggs seemed to be oviposited at/near the calyx than in the north. As shown in Tab. 3, differences in egg placement on apples between localities in northern Switzerland and southern Ticino are significant. Even when numbers in holes/tunnels at the calyx are added to all holes, it does not equalize the distribution over apples between the two areas as occurred in comparisons of *C. amoena* oviposition on parasitized apples in Michigan and Virginia (BAND, 1994).

Rhynchites females puncture the developing fruit to lay one or more eggs; larvae feed on the seeds and may pupate either in the fruit or in the ground (KOTTE, 1958). Hence a weevil larva most likely creates a tunnel and hole at the side of an apple. Codling moth larvae typically enter apples at the stem or calyx and tunnel along the core. Two generations per year may occur in southern Ticino. MANI *et al.* (1997) showed that only one generation a year occurs in eastern Switzerland. This is probably the case in the Zürich and Aargau areas also, indicating that weevil larvae do the most damage to apples in mid-July in northern Switzerland.

As shown in Tab. 4, *C. amoena* emerged from eggs collected from apples at Hönggerberg, Purasca, Castel San Pietro, from chestnuts collected at Purasca, from acorns collected in the Bolle di Magadino and from the one plum with a single egg at Castelrotto. The fact that there were more eggs than resulted in adult *C. amoena* is in agreement with findings in both Michigan and Virginia (BAND, 1988a, 1996). *C. amoena* females lay unfertilized eggs, while some eggs had already hatched but larvae could not be found within the interior of the somewhat relatively dry apples.

Tab. 5. Drosophilids netted in the Val Blenio, Ticino, 17–20 July 1998.

Species Altitude m	Alteniga 870	Corzoneso 670	Acquarossa 530	Total
<i>C. amoena</i>		1	1	2
<i>C. fuscimana</i>			1	1
<i>D. bifasciata</i>			1	1
<i>D. busckii</i>	1		1	2
<i>D. deflexa</i>	1			1
<i>D. fenestrarum</i>			27	27
<i>D. funebris</i>	39	10	3	52
<i>D. helvetica</i>	24	16	4	44
<i>D. histrio</i>			2	2
<i>D. immigrans</i>	93	66	161	320
<i>D. kuntzei</i>	137	150	686	973
<i>D. limbata</i>			63	63
<i>D. melanogaster</i>			2	2
<i>D. miki</i>			1	1
<i>D. nigrosparsa</i>	1			1
<i>D. obscura</i>	61	28	88	177
<i>D. phalerata</i>	208	143	473	824
<i>D. simulans</i>			3	3
<i>D. subobscura</i>	49	98	38	185
<i>D. testacea</i>	393	369	1924	2686
<i>D. transversa</i>	1		2	3
<i>D. tristis</i>	4	3	2	9
<i>D. sp.</i>			16	16
<i>S. pallida</i>	7	2	12	21
Total	1019	886	3511	5416

In Virginia for the years 1985 and 1986, adult emergence from the number of *C. amoena* eggs counted in apples, 935, was 11.8 % (BAND, 1988a). Emergence from the 273 *C. amoena* eggs counted in July 1998, 10.6 %, compares favorably despite the fact that eggs had been removed from initial substrates and transferred to artificial medium.

Tab. 5 confirms that *C. amoena* was present among drosophilids at higher elevations in the Valle Blenio region as on the lower slopes of the mountains about Cevio in the Maggia Valley (BAND *et al.*, 1998). No emergence occurred from the chestnut containing *C. amoena* larvae and pupae in the mixed forest at the site where *C. amoena* was netted. Apples along the slopes lacked evidence for *C. amoena*, and the agricultural region in the delta was too sunny. One *D. simulans* male and one *D. immigrans* male emerged from apples collected in this area, although more *D. immigrans* eggs were counted. Nevertheless, *C. amoena* also came to bait in the wooded area above Acquarossa, as shown in Tab. 5.

In contrast to WINSTON (1957) that *C. amoena* in acorns were feeding on moulds *Penicillium* and *Fusarium*, yeasts were present in 40.5 %, moulds in 70 % of 37 apples with *C. amoena* eggs and larvae. At present we cannot say if there are differences in microbial content between apples with *C. amoena* eggs in northern Switzerland and southern Ticino.

DISCUSSION

BURLA & BÄCHLI (1992) pointed out that *C. amoena* entered Europe as a domestic species on apples, and had spread widely in Eastern and Central Europe after its initial capture in 1975. MACA & BÄCHLI (1994) suggested its rapid spread was due to its ability to exploit an open ecological niche. Its nut breeding capacity (acorns, chestnuts, hazelnuts) accounted for its ability to invade forests especially in Canton Ticino (BURLA & BÄCHLI, 1991, 1992; BAND *et al.*, 1998) although European forests have largely been closed to insect invaders (NIEMALÄ & MATTSON, 1996). BAND (1996) argued that *C. amoena*'s ability to exploit parasitized unripe and ripe fruits for breeding may also account for its spread in Europe, a niche which enabled it to act as a domestic species in the United States. Evidence obtained in July 1998 shows that this is precisely the open niche it entered in Switzerland when true pests attacking developing apples make parasitized fallen apples available for oviposition in early to mid-summer.

The fact that it continues to exploit parasitized nuts and fruits in Europe as in the United States and is adapted to small population size may also account for its successful establishment in Europe. The variety of substrates in which it can breed lessens its chance of extinction due to crop failure in a given year. Whereas parasitized acorns were plentiful in the Bolle di Magadino in July 1997 (BAND *et al.*, 1998), they were few in number in July 1998. Whereas a cold spring had destroyed the apple crop in some places in 1997 and hindered true pest oviposition, this niche was widely available in 1998. Indeed, it would appear that *C. amoena* has reestablished its affinity for breeding in frassy fruits created by weevil and codling moth larvae. It may also have entered substrates as yet unknown because the 597 *C. amoena* coming to bait in the Bolle di Magadino in June 1995 suggests multiple breeding sites there, not just acorns; domestic and wild fruits are not plentiful, nor are chestnuts.

Given the extent of *C. amoena*'s presence in southern Ticino, its existence in northern Italy is highly likely although this species has not been reported in that country. It has recently been reported in France (WITHERS & ALLEMAND, 1998). *C. amoena* may not spread as rapidly in western Europe as did *D. melanogaster* or more recently *D. simulans* but given time it may become just as widespread.

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