Presence of auchenorrhyncha known or suspected vectors of phytoplasmas in vine growing areas of the Aosta Valley

Présence d' auchenorrhyncha connus ou potentiels vecteurs de phytoplasmes dans les surfaces viticoles de la Vallée d'Aoste

Presenza di auchenorrinchi noti o potenziali vettori di fitoplasmi in aree viticole della Valle d'Aosta

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Abstract
Grapevine yellows are a serious problem for Italian viticulture. The most threatening is currently Flavescence dorée, which is caused by *Candidatus* Phytoplasma vitis 16Sr-V (subgroups C and D) and is transmitted by *Scaphoideus titanus* Ball (Homoptera Cicadellidae), a monophagous species from the nearctic region. On the other hand, Bois noir (BN), a disease which is commonly widespread in Italy, seems to be less epidemic: it is caused by *Candidatus* Phytoplasma solani 16Sr-XII, and is transmitted by *Hyalesthes obsoletus* Signoret (Homoptera Cixiidae), a palearctic planthopper which lives on nettle (*Urtica dioica* L.) and only occasionally feeds on grapevine, as an adult. Currently, in the Aosta Valley the FD has not yet been detected, whereas BN is present in many areas. This research investigates the presence and abundance of some insect vectors of phytoplasmas to grapevine in the Aosta Valley.
Studies were conducted during 2002-2004 in 14 vineyards, each of them located in a different district of the Aosta Valley: seven in the high and seven in the low valley. Some of them were subject to Integrate Pest Management (IPM), whereas others were not. Yellow sticky traps were used to investigate the presence of *S. titanus* and *H. obsoletus*. Three traps per vineyard were placed into the vine canopy and other three in nearby nettle beds, and changed every 10 days from the beginning of July to the end of September. Sampling was also made by using a sweep net. Insects were identified and sexed in the laboratory, using a stereomicroscope. PCR analyses were performed to detect the presence of 16Sr-V and 16Sr-XII phytoplasmas in *S. titanus* and *H. obsoletus* adults, respectively.
The presence of *S. titanus* was quite scarce in the high valley, where 45.4, 26.8 and 11.3 adults per vineyard were caught. On the other hand, in the low valley this pest was present with high levels of population: 252.9, 114.4, and 126.3 adults per vineyard. The flight peak occurred approximately in the middle of August, and more males than females were captured.
H. obsoletus was abundant both in the high and in the low valley, but only in nettle beds, where 18.5, 6.8, and 22.2 adults were captured, whereas few specimens were caught on the vine canopy. PCR essays on *S. titanus* showed no presence of *Candidatus* Phytoplasma vitis; on the other hand, many *H. obsoletus* (up to 84%) were found to be positive to *Candidatus* Phytoplasma solani.
Finally, *Anoplotettix fuscovenosus* (Ferrari) (Homptera Cicadellidae), a suspected vector of phytoplasmas, was found to be widespread both in the high and the low valley, with 60.4, 15.9, and 19.7 individuals per vineyard.

Résumé
Les jaunissements de la vigne représentent un problème sérieux pour la viticulture italienne. Parmi eux, actuellement, le plus inquiétant est sans doute la Flavéscence dorée (FD), qui est provoquée par *Candidatus* Phytoplasma vitis 16Sr-V (sous-groupes C et D) et qui est transmise par *Scaphoideus titanus* Ball (Homoptera Cicadellidae), espèce étroitement inféodée à la vigne et originaire de l'Amérique du nord. Le Bois noir (LN), maladie très répandue en Italie, semble moins épidémique; elle est causée par *Candidatus* Phytoplasma solani 16Sr-XII et elle est transmise par *Hyalesthes obsoletus* Signoret (Homoptera Cixiidae), espèce paléartique, inféodée à l'ortie (*Urtica dioica* L.), se nourrissant seulement au stade imaginal et occasionnellement aux dépens de la vigne. Actuellement, en Vallée d'Aoste, la FD n'a pas encore été détectée, tandis que le Bois noir a été signalé en plusieurs lieux.
Ce travail de recherche se propose d'enquêter sur la présence et la quantité de certains insectes vecteurs de maladies à phytoplasmes de la vigne en Vallée d'Aoste.
Les études ont été conduits pendant les années 2002-2004 dans 14 vignobles, répartis en autant de communes du Val d'Aoste: de ces 14 vignobles, sept se trouvent dans la Haute Vallée et sept dans la Basse Vallée. Certains vignobles étaient conduits selon les méthodes de la lutte intégrée. Pour déterminer la présence de *S. titanus* et *H. obsoletus* on a employé des pièges collants jaunes. Trois pièges par vignoble ont été placées sur la partie aérienne de la plante et trois là où il y avait des orties; les pièges ont été remplacées chaque dix jours à partir du mois de juillet jusqu'à la fin de septembre. Les échantillonnages ont été même réalisés avec l'aide d'un filet entomologique.
Dans les laboratoires, avec un stéréomicroscope, on a identifié et sexé les insectes capturés. Un certain nombre d'individus adultes de *S. titanus* et *H. obsoletus* ont été soumis à des analyses moléculaires (PCR) pour relever la présence de phytoplasmes attribuables aux groupes 16Sr-V et 16Sr-XII.
S. titanus s'est révélé peu nombreux dans la Haute Vallée d'Aoste, où l'on a capturé 45,4, 26,8 et 11,3 adultes par vignoble; au contraire, dans la Basse Vallée, il a atteint des niveaux de population élevés: 252,9, 114,4 et 126,3 adultes par vignoble. Le sommet du vol s'est avéré à peu près vers la mi-août, et on a capturé plus de mâles que de femelles.

H. obsoletus s'est révélé abondant soit au nord-ouest soit au sud-est de la région, mais là seulement où il y avait des touffes d'orties on y a capturé 18,5, 6,8, et 22,2 adultes, tandis que peu d'individus ont été récoltés sur la couronne des plantes. Les analyses moléculaires (PCR) conduites sur *S. titanus* n'ont pas mis en évidence la présence de *Candidatus* Phytoplasma vitis; au contraire, un grand nombre d'adultes de *H. obsoletus* (jusqu'à 84%) s'est avéré positif sur *Candidatus* Phytoplasma solani.
Enfin, soit dans la Haute que dans la Basse Vallée d'Aoste, *Anoplotettix fuscovenosus* (Ferrari) (Homptera Cicadellidae), vecteur potentiel de phytoplasmes, s'est révélé répandu avec 60,4, 15,9, et 19,7 individus par vignoble.

Riassunto
I giallumi della vite sono un serio problema per la viticoltura italiana. Il più preoccupante è attualmente la Flavescenza dorata (FD), che è causata da *Candidatus* Phytoplasma vitis 16Sr-V (sottogruppi C e D) ed è trasmessa da *Scaphoideus titanus* Ball (Homoptera Cicadellidae), una specie monofaga originaria dell'areale neartico. Il Legno nero (LN), malattia comunemente diffusa in Italia, sembra meno epidemico: è causato da *Candidatus* Phytoplasma solani 16Sr-XII, ed è trasmesso da *Hyalesthes obsoletus* Signoret (Homoptera Cixiidae), una specie paleartica infeudata all'ortica (*Urtica dioica* L.) che si nutre solo occasionalmente da adulto a spese della vite. Attualmente, in Valle d'Aosta la FD non è ancora stata ritrovata, mentre il LN è presente in diverse aree. Questa ricerca ha lo scopo di indagare sulla presenza ed abbondanza di alcuni insetti vettori di fitoplasmi alla vite in Valle d'Aosta.
Le ricerce sono state condotte nel periodo 2002-2004 in 14 vigneti, distribuiti in altrettanti comuni della Valle d'Aosta: sette si trovavano nell'Alta Valle e sette nella Bassa Valle. Alcuni erano gestiti con metodi di lotta integrata. Sono state utilizzate trappole adesive gialle per rilevare la presenza di *S. titanus* e *H. obsoletus*. Tre trappole per vigneto sono state sistemate sulla chioma della vite e tre in aree con ortiche, e sostituite ogni 10 giorni dall'inizio di luglio alla fine di settembre. I campionamenti sono stati fatti anche usando un retino entomologico. Gli insetti catturati sono stati identificati e sessati in laboratorio, usando uno stereomicroscopio. Sono state eseguite analisi PCR per rilevare la presenza di fitoplasmi dei gruppi 16Sr-V e 16Sr-XII, rispettivamente in adulti di *S. titanus* e *H. obsoletus*.
La presenza di *S. titanus* è risultata abbastanza scarsa nell'Alta Valle, dove sono stati catturati: 45,4, 26,8 e 11,3 adulti per vigneto. Invece, nella Bassa Valle è risultato presente con livelli di popolazione elevati: 252,9, 114,4 e 126,3 adulti per vigneto. Il picco di volo è avvenuto approssimativamente alla metà di agosto, e sono stati catturati più maschi che femmine.
H. obsoletus è risultato abbondante sia in Alta che in Bassa Valle, ma solo entro formazioni di ortiche, dove sono stati catturati 18,5, 6,8 e 22,2 adulti, mentre pochi individui sono stati raccolti sulla chioma della vite. Le analisi di PCR condotte su *S. titanus* non hanno mostrato presenza di *Candidatus* Phytoplasma vitis; al contrario, molti adulti di *H. obsoletus* (fino all' 84%) sono risultati positivi al *Candidatus* Phytoplasma solani.
Infine, sia in Alta che in Bassa Valle è risultato diffuso *Anoplotettix fuscovenosus* (Ferrari) (Homptera Cicadellidae), un potenziale vettore di fitoplasmi, con 60,4, 15,9 e 19,7 individui per vigneto.

Key words: *Scaphoideus titanus*, *Hyalesthes obsoletus, Anoplotettix fuscovenosus,* FD, BN

Introduction

Among the 15 known ribosomal groups of phytoplasmas, two are known to cause Grapevine Yellows (GY) in Italy: Elm Yellows, which include Flavescence dorée (FD, caused by "*Candidatus* Phytoplasma vitis" or 16SrV subgroups C and D) transmitted by the nearctic leafhopper *Scaphoideus titanus* Ball (Homoptera Cicadellidae), and Stolbur, which includes Bois Noir (BN, caused by "*Ca.* Phytoplasma solani" or 16SrXIIA) transmitted by the palearctic planthopper *Hyalesthes obsoletus* Signoret (Homoptera Cixiidae) (Boudon-Padieu, 2003; Botti and Bertaccini, 2005).
Nowadays, FD is reported in Southern France and Italy (Boudon-Padieu, 2003), Northern Spain (Battle *et al.,* 2000), Switzerland (Anonymous, 2004) and Serbia (Duduk *et al.*, 2003). In Italy it is widespread in all Northern regions apart from Aosta Valley (Borgo *et al.,* 2005); the situation seems to be more patchy in Central Italy: there are reports in Northern Tuscany and Umbria (Borgo *et al.,* 2005), and Marche (Romanazzi *et al.,* 2005). On the other hand, BN is widespread in Europe and Italy, including the Aosta Valley (Borgo *et al.,* 2005).
S. titanus was first introduced into Europe in the 1950s' in Southern France, and is present in Italy since 1963 (Vidano, 1964). It is now settled in Southern France, Corsica, Switzerland, Italy, Northern Spain, Northern Portugal, Slovenia, Croatia and Serbia (Alma, 2004). This pest is settled in all Northern Italian regions, including the Aosta Valley, whereas it is more localized in Central and Southern Italy (Alma, 2004). It lives only on grapevine and is univoltine: eggs overwinter under the bark of two-year old wood, and hatching usually starts in May and ends in July, with nymphs feeding on lower leaves; adults are present from July to October (Vidano, 1964). Its flight activity is crepuscular and quite restricted to the vine's canopy (Lessio and Alma, 2004a, b).

H. obsoletus on the contrary, is a native palearctic species widespread in Europe. It is not a grapevine specialist: nymphs live and overwinter on roots of dicotyledonous herbaceous plants, and adults feed on leaves of the same plants although they can occasionally shift onto grapevine transmitting BN phytoplasmas. In Italy, *H. obsoletus* is known to live mainly on *Urtica dioica* L. (Alma *et al.,* 2002), whereas in Central Europe its host range includes also *Convolvulus arvensis* L., *Ranunculus bulbosus* L., *Calystegia sepium* (L.) and *Cardaria draba* L. (Sforza *et al.,* 1998; Langer *et al.,* 2003), and *Vitex agnus-castus* L. in Israel (Sharon *et al*., 2005).
Other palearctic homopterans besides *H. obsoletus* are suspected to be BN vectors. Among Cixiidae, *Reptalus panzeri* (Löw) has been reported to be associated to Stolbur phytoplasmas in Hungary (Palermo *et al.,* 2004). Another suspected vector is *Anoplotettix fuscovenosus* (Ferrari) (Cicadellidae), whose adults feed on broad-leaved trees and shrubs (including grapevine) where eggs are laid, while juveniles feed on dicotyledonous herbaceous plants; this species is reported to transmit phytoplasmas in laboratory conditions, but its role as a vector in the open field is still to be proved (Alma, 1995).
Although in the Aosta Valley FD is not yet present, there are no data reported on the spread of *S. titanus*; moreover, there is little knowledge on the diffusion of BN and of its known and potential vectors. The aim of this research was therefore to monitor auchenorrhyncha that are known or suspected vectors of Grapevine Yellows' phytoplasmas in the Aosta Valley.

Materials and methods

Field sampling
Data were collected in 2002-2004, in 14 vineyards located in different districts of the Aosta Valley: seven in the high valley (from Aosta to Morgex) and seven in the low valley (from the border with Piedmont to Aosta). The population dynamics of different homopteran species was studied using Rebell® Amarillo (Andermatt Biocontrol AG, Switzerland) sticky traps: three traps per vineyard were placed in a diagonal pattern in the vine canopy. When nettle (*U. dioica*) was present nearby, other three traps were placed directly inside nettle beds. Traps were changed every ten days from the beginning of July to the end of September. In the laboratory, trapped adult homopterans were unglued with a drop of benzene and then determined and divided by sex using stereomicroscope. Screening was particularly accurate for three species: *S. titanus*, *H. obsoletus* and *A. fuscovenosus*. Specimens were preserved inside eppendorf tubes in acetone before analysis. Moreover, other samplings were made with sweep-net in the vine canopy and in nettle beds; in the laboratory specimens were frozen and preserved at -20°C before molecular analysis. During the insect samplings the plants were inspected and grapevine yellows symptoms recorded. To confirm the presence of phytoplasmas, leaf samples from 10 symptomatic plants were taken during summer and analysed by molecular means.

Laboratory analyses
S. titanus specimens were subjected to molecular analysis to detect the presence of "*Ca.* Phytoplasma vitis". Total DNA was extracted following a protocol specifically adapted to leafhopper phytoplasma vectors (Marzachì *et al.,* 1998); samples where then processed with direct PCR using fAY/rAY specific primers, which can detect FD in insect vectors with the same reliability of nested PCR (Marcone *et al.,* 1996). In case of doubtful results, DNA was first amplified with F2/R2 universal primers (Lee *et al*., 1993), and then in nested PCR with F1(V)/R1(V) specific primers (Lee *et al.,* 1994); amplification products were analyzed with electrophoresis in 1-1.5% agarosium gel. DNA from *H. obsoletus* and *A. fuscovenosus* specimens was extracted following the same procedure, and then analyzed with M1/P8 primers for detection of "*Ca.* Phytoplasma solani" (Marzachì *et al.,* 2000). In case of doubtful results, PCR amplicones were analyzed in Dot-Blot with the riboprobe pTS_1 224 (Dot-Blot on *A. fuscovenosus* is still in process) (Marzachì *et al.,* 2000). Reaction and cycling conditions were as in the original papers.
Midribs of leaves (1 g) from symptomatic plants were used for DNA extraction according to the phytoplasma-enrichment procedure described by Ahrens and Seemüller (1992). The plant DNA was analyzed for the presence of "*Ca.* Phytoplasma vitis" and "*Ca.* Phytoplasma solani" following the same procedures described for the insects.

Statistical analysis
Data from sticky traps were analyzed via two-way ANOVA to detect significant differences due to the zone (high or low valley) and the year of sampling. Linear regression was also calculated to detect a trend between the altitude and the captures of *S. titanus* and *A. fuscovenosus.*

Results

Presence of vectors and population dynamics

Populations of *S. titanus* were quite scarce in the High Valley, apart from vineyard no. 7 located almost on the border between the two zones, whereas in the Low Valley they were sometimes high locally (Tab. 1); anyway, significant differences were found between population levels of the two zones (F=43.6, P<0.001). On the other hand, no differences were detected between different years (F=2.53, P=0.08), and no significant interaction was found between year and zone (F=1.15, P=0.32). In some cases, many individuals were captured on traps placed in nearby nettle beds (Tab. 2). The presence of *S. titanus*, significantly decreased with altitude (F=20.22, R^2=0.67, P<0.001) (Fig. 1). *S. titanus* showed a flight peak at the middle of August, and more males than females were captured (Fig. 2).

Table 1. Population levels of *Scaphoideus titanus* Ball, *Hyalesthes obsoletus* Signoret and *Anoplotettix fuscovenosus* (Ferrari) observed on traps placed on grapevine. Alt.: altitude (m asl); P.: position (H: high valley; L: low valley).

N	**Location**	**Alt.**	**P.**	***S. titanus***			***H. obsoletus***			***A. fuscovenosus***		
				2002	2003	2004	2002	2003	2004	2002	2003	2004
1	Aosta	583	H	1	0	7	2	0	0	139	17	7
2	St. Pierre	731	H	0	0	4	0	0	0	54	8	7
3	La Salle	1001	H	0	0	4	0	0	0	242	37	78
4	Morgex	923	H	0	0	4	0	0	0	123	31	74
5	Aymavilles	640	H	10	6	0	1	0	2	44	7	7
6	Aymavilles	640	H	4	3	2	0	3	0	34	24	25
7	St. Christophe	619	H	303	179	58	1	0	0	3	4	3
8	Nus	529	L	124	46	24	11	0	0	23	20	7
9	Chambave	486	L	694	148	113	0	0	0	33	5	5
10	Chatillon	549	L	100	15	17	0	0	1	41	6	8
11	Montjovet	406	L	62	27	56	1	1	0	21	12	9
12	Issogne	387	L	348	325	504	2	0	1	3	2	20
13	Arnad	361	L	441	240	170	4	1	2	29	9	20
14	Donnas	322	L	1	0	0	4	0	0	57	57	6

Table 2. Population levels of *Scaphoideus titanus* Ball, *Hyalesthes obsoletus* Signoret and *Anoplotettix fuscovenosus* (Ferrari) observed on traps placed on nettle. Alt.: altitude (m asl); P.: position (H: high valley; L: low valley).

N	**Location**	**Alt.**	**P.**	***S. titanus***			***H. obsoletus***			***A. fuscovenosus***		
				2002	2003	2004	2002	2003	2004	2002	2003	2004
3	La Salle	1001	H	0	0	1	45	1	40	91	17	107
7	St. Christophe	619	H	263	138	1	1	0	0	16	4	0
11	Montjovet	406	L	21	3	0	14	15	5	1	0	0
13	Arnad	361	L	573	214	95	14	11	44	20	11	15

H. obsoletus was captured mainly near *U. dioica*, and few specimens were present on the grapevine canopy (Tabb. 1-2). The flight peak occurred at the end of July, and more males than females were captured (Fig. 3). No significant differences of captures in nettle were found between zones (F=5.2, P=0.10), nor between years (F=1.74, P=0.31), and no significant interaction was evident between zone and year (F=1.04, P=0.50), but stations with nettle were probably too few.

Many individuals of *A. fuscovenosus* were captured on grapevine, and many of them were present also on nettle (Tabb. 1-2). Captures of this species significantly increased with altitude (F=5.00, R^2=0.29, P=0.03) (Fig. 1). The flight peak occurred at the middle of July and more females were captured (Fig. 4). Differences were found between zones (F=4.91, P=0.03) and years (F=6.4, P=0.004), but with no interaction (F=1.13, P=0.33).

Presence of phytoplasmas

None of the 306 *S. titanus* individuals tested during the three-year sampling period were found to be FD-positive with either of the molecular techniques. On the other hand, 25 *H. obsoletus* specimens out of 178 tested were BN-positive with M1/P8 (14%), whereas with Dot-Blot positive individuals were 76 (43%). All BN-positive specimens came from the same vineyard (no. 13), where many vine plants presented GY symptoms and were found to be BN-positive. Preliminary tests with M1/P8 primers on 52 *A. fuscovenosus* adults showed no presence of BN.

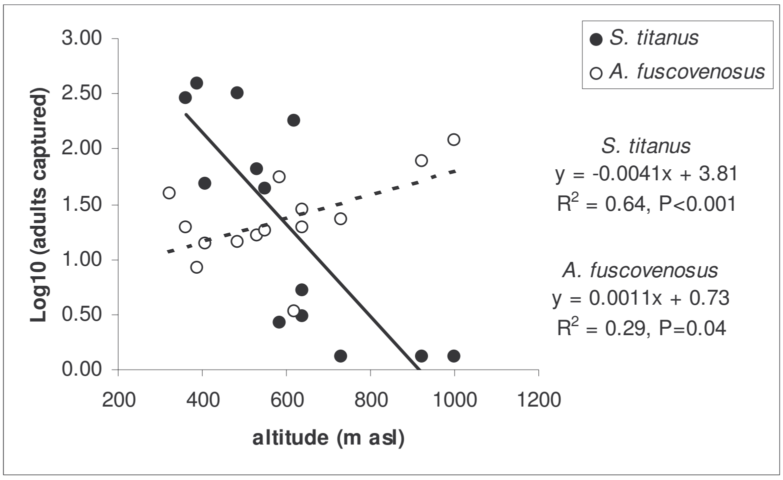

Figure 1. Linear regression between the altitude and the presence of *Scaphoideus titanus* Ball (solid line) and *Anoplotettix fuscovenosus* (Ferrari) (dashed line).

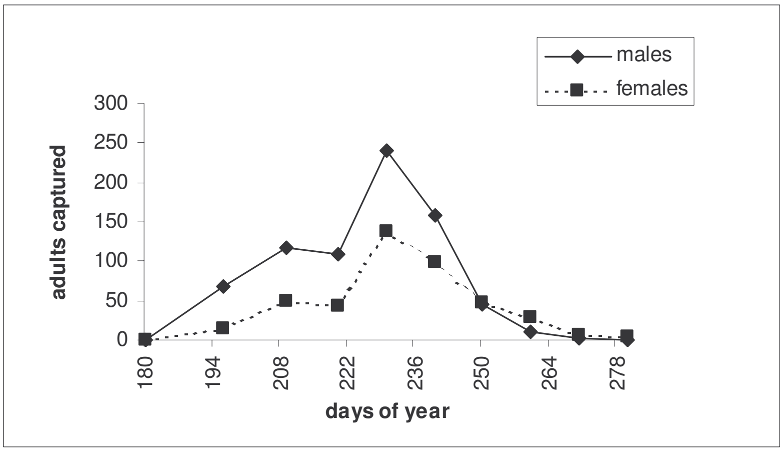

Figure 2. Population dynamics of *Scaphoideus titanus* Ball on grapevine in the Aosta Valley (mean per year). *Sex ratio* (m/f): 1.75.

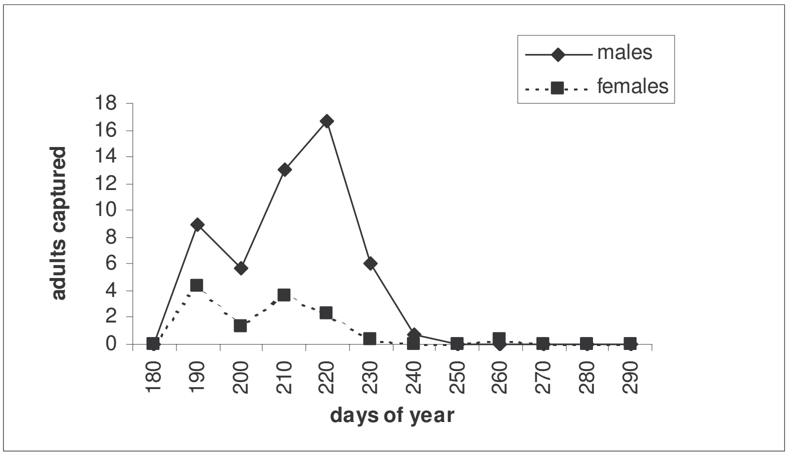

Figure 3. Population dynamics of *Hyalesthes obsoletus* Signoret on nettle in the Aosta Valley (mean per year). *Sex ratio* (m/f): 4.14.

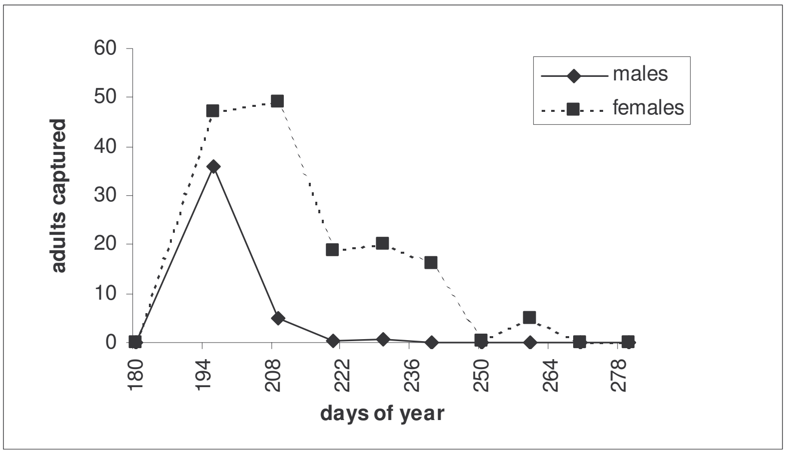

Figure 4. Population dynamics of *Anoplotettix fuscovenosus* (Ferrari) on grapevine in the Aosta Valley (mean per year). *Sex ratio* (m/f): 0.26.

Discussion

S. titanus was found to be widespread in the low valley, whereas its populations were quite small in the high valley; as a positive relationship was found between altitude, it seems that this pest is not capable of colonizing mountain vineyards over 600 m above sea level. However, in the High Valley this species has increased during 2004. On the other hand, population levels were very high in the low valley and this could be a problem if FD is going to spread also in the Aosta Valley, which is likely since it is already present in many neighbour countries. An exception was noted in vineyard no. 14, where insecticides are currently applied against grapevine moths. The flight peak of *S. titanus* in this region comes almost one month later than in Piedmont (Lessio and Alma, 2004b), and this aspect is to be considered if insecticides will need to be applied. The capture of many specimens on traps placed in nettle beds is understandable because of the strong wind and because, in man cases (vineyards no. 7 and 13), nettle was very close to vine rows. Moreover, *S. titanus* was never found on *U. dioica* during samplings with sweep-net.

H. obsoletus was not so dependent on altitude, however it is not widespread, being linked to the presence of its main host plant in Italy, *U. dioica*: the same results were obtained in Piedmont (Alma *et al.,* 2002), and in Central Italy (Milanesi *et al.,* 2005). The percentage of BN-positive individuals in the Aosta Valley is sometimes very high, concordant with the results of other researches (Maixner and Reinert, 2000); however, few individuals were captured on the vine canopy, that is consistent with the results of another research (Nicoli Aldini *et al.,* 2003); it is likely therefore that other vectors of BN phytoplasmas are present.

A. fuscovenosus was found all along the Aosta Valley, although a slight influence in altitude was detected, probably because of differences in the abundance of its herbaceous host plants. Many more females than males were captured in the grapevine canopy, because eggs are laid on vine (Alma, 1995). To date, there is no evidence that *A. fuscovenosus* could harbor BN-phytoplasmas, but more information is needed. For example, in Central and Southern Italy, some individuals of *A. putoni* Ribaut, which life cycle is still not known, recently were found to be positive to BN phytoplasmas (Bagnoli *et al.,* 2005).

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