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Studies on the sterile male technique as a means of control of Adoxophyes orana (Lepidoptera, Tortricidae). 5. Release trials

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Abstract

In release trials with sterile males of *Adoxophyes orana* in an apple orchard of 1.4 ha in 1971 and in an other orchard of 1.9 ha in 1973 and 1974 a marked decrease in the population of *A. orana* was observed. Release of sterile males seemed to cause the effect. Immigration of moths, usually not more than a few hundreds, did not disturb the programme but would obviously prevent eradication and control at low level. Other tortricids increased in the absence of chemical control but less competition from *A. orana* might also offer some explanation. The other tortricids were responsible for up to 9% damage among apples. Consequently the sterile male method specifically against one pest does not result in an important reduction in the number of sprays and a specific control method is not always the solution to pest problems in integrated control.

Introduction

For an integrated control programme in apple orchards, a selective control method for *Adoxophyes orana* is urgent. This pest is usually controlled chemically with azinphosmethyl, carbaryl or other broad-spectrum insecticides. These interfere with biological control agents like predators of red spider mite, *Panonychus ulmi*. A possible solution could be the specific sterile male method.

The practicallity of this technique can only be judged from actual release trials in the field. These trials will help to finally synthesize and evaluate all previous analytical experimental work.

As a first requirement a method for mass rearing on a scale sufficient for treatment 1–2 ha was developed (Ankersmit and Van der Meer, 1973; Ankersmit et al., 1977). This method made possible a production of 5000–10000 moths per day.

Sterilization by irradiation was studied by Snieder et al. (1973). They showed that doses of 25 krad X-rays to males reduced egg hatch with 90–95% in eggs laid by untreated females, to which they were mated. The few offspring were mostly sterile (Snieder and Ter Velde, 1975). Females were completely sterilized by 25 krad.

Mating competitiveness of irradiated reared males was found to be reduced to about 0.6 of that of wild moths (Denlinger et al., 1973).

Migration was expected to be of minor importance when the orchards, used for the release trials were surrounded by open pasture (Barel, 1973).

The phenology of the pest was well studied by Van Frankenhuyzen and De Jong

(1964). Two well separated generations were always observed, flights occurring in June and August – September.

Census methods had to be developed as usually only population levels have been studied by counting the larvae on a certain number of clusters or shoots. In our trials, we needed absolute population estimates. From the available data, we expected population densities in June of about 1000 moths per ha in chemically treated orchards. If so, release in ratios of about 20:1 should reduce the population in one year to about 100 (De Jong et al., 1971). In view of the constant small immigration that could be expected, complete eradication seemed senseless. The best one could hope for was that the rate of multiplication and immigration could be matched by a regular release of a few sterile moths.

Since selective control of *A. orana* was meant to avoid chemical control with broad-spectrum insecticides, the question arose as to what would happen to the numbers of usually secondary insect pests, among them many other leaf-roller species (Tortricidae), when these were no longer subject to insecticide treatments.

The effectiveness of the method and problems were studied in two release trials, one in 1971 near Overberg (Utrecht) and the other in 1973 and 1974 near Lienden (Betuwe).

Material and methods

A. orana was reared as described by Ankersmit et al. (1977). In the first trial, we used the diet with agar as gelating agent; in the second trial, we used the diet based on lucerne meal. Moths were irradiated with 25 krad at room temperatures in small refrigerator boxes from a ^{60}Co source at the 'Pilot Plant for Food Irradiation' at Wageningen. Exposure time was 20–25 min. The effect of irradiation was checked by D. Snieder and H. J. ter Velde, Institute for Atomic Energy in Agriculture, Wageningen, with samples of 10 to 20 females and males taken just after irradiation. The average egg-hatch from matings of these irradiated males with unirradiated females was 9.3% (range 1–26%) in 1973 and 5.4% (0.5–17.8%) in 1974. As the resulting moths from these matings were largely sterile this hatch seemed sufficiently low (Snieder and Ter Velde, 1975). Only moths one day old or moths that had been stored at 2–4 °C for one day after collection from the emergence boxes were used. As females were completely sterilized (Snieder et al., 1973), they were released with the males. Sex ratio was about 1:1. The number of released moths was estimated by weighing (Ankersmit et al., 1977).

All released moths were marked. In the trial near Overberg, Calco oil Red D (American Cyanamid Company, Bound Brook N.J.) was added to the agar diet (Barel, 1973). In the trial near Lienden Rotor dyes (ICI, London) were dusted upon the moths a few hours before irradiation. The dyes were prepared by carefully mixing equal amounts of the dye and tannin in a mortar. By using different colours for each day of release, the age of the recaptured moths could be established. Effect of irradiation on quality of the moths was studied in four small trials. Equal numbers of irradiated and unirradiated moths (25 krad) were released and recaptured with pheromone traps' All moths were marked (Table 1). A decrease was observed in recapture of irradiated moths. This recapture further decreased at doses of 35 and 50 krad. This justifies our use of the lowest possible dose in the release trials. The

Table 1. Number of recaptured irradiated and unirradiated released reared males.

Date of release	Number released per group	Number recaptured			
		untreated	25 krad	35 krad	50 krad
12 September 1973	94	32	22		
14 September 1973	86	27	20		
12 July 1974	100	37	35		
10 September 1974	100	44	35	30	22
Total		140	112		

Tabel 1. Aantal teruggevangen bestraalde en onbestraalde losgelaten gekweekte mannetjes.

results indicated that flight activity of irradiated moths might be reduced to 0.8 of that of unirradiated moths.

The powder dusted upon the moths for marking might interfere with their ability to find pheromone traps. Recapture data (Table 2) indicated a reduction in recapture of marked moths to about 0.75 of that of unmarked. The results however varied a great deal.

Moths were released by walking with open refrigerator boxes through the orchard. As each box contained about the same number of moths, and per row of trees the same number of boxes was used, an even distribution throughout the orchard was achieved. Moths were released usually twice but sometimes thrice per week in order to adjust the release numbers to the number of native moths according to recapture data in light or pheromone traps.

The orchard near Overberg consisted of 0.9 ha spindle-form trees of 11 years old (cv. Cox's Orange Pippin, Golden Delicious, Benoni) and 0.5 ha newly planted Schone van Boskoop and Cox's Orange Pippin. The orchard near Lienden was 1.9 ha with spindle-form trees of 9 years old (cv. Cox's Orange Pippin, James Grieve, Jonathan and Benoni). In both orchards, only the normal fungicide treatments with dinocap and captan were applied as well as cyhexatin for red spider control. In the trial near Lienden, rosy apple aphid was controlled with pirimicarb, which has no effect upon leafrollers.

Before the trials, populations were reduced by a preblossom spray. In the trial

Table 2. Number of recaptured and unmarked released males.

Date of release	Number released per group	Number recaptured	
		marked	unmarked
21 September 1973	100	8	12
9 July 1974	100	33	38
30 July 1974	100	19	38
11 September 1974	100	51	55
Total		111	143

Tabel 2. Aantal teruggevangen gemerkte en ongemerkte losgelaten mannetjes.

near Overberg, two sprays with methylparathion were applied in April 1971 and in the trial near Lienden one spray with diazinon on 17 april 1973 and one with parathion on 14 May 1973.

In the trial near Overberg, moths were recaptured in six Robinson light traps with 125-Watt HPL lamps. In the trial near Lienden, we used 21 pheromone traps of the type described by Minks and Voerman (1973), each baited with 500 μg pheromone and evenly distributed over the orchard. The ratio between recaptured moths and unmarked native moths was expected to give some indication of the number of moths to be released.

Larvae were counted on whole trees. As most suitable moments, the beginning of May and the end of July were selected. Most larvae are then nearly full-grown and are easily found. This count gave some indication about the size of the next flight as mortality in the pupal stage seemed small. For the identification, all collected larvae were reared to adult on artificial diet. Larvae that failed to grow to adult were identified as larvae by the method of Evenhuis and Vlug (1972) or as pupa (Evenhuis et al., 1973). Parasites were identified by H. Vlug (Institute of Phytopathological Research, Wageningen) or by us with the key of Evenhuis (1974). Counts were also made in September but then only small second and third instar larvae were present. These counts must be considered less exact than those in May and July as larvae were hard to find. Some of the larvae may already have entered diapause in a hibernaculum on the stem, and would be overlooked. However the counts helped to forecast the size of the next year's population. This information could be used for planning the scale of mass rearing.

Results

The number of released males during the actual flight period is given in Table 3 except for August 1973 when 37000 males were released. Between the two flights, about 1000 males were released per week. Releases were continued in September but their numbers were omitted, as we found later that flights in that period were unlikely

Table 3. Capture and release data of the trials near Overberg and Lienden. All figures refer to numbers of males in the whole orchard.

Flight period	Number caught		Ratio native: released	Number calculated		Fraction captured	
	native	released		native	released	native	released
Overberg							
June 1971	244	929	1:4	100	41000	2.44	0.02
August 1971	423	5368	1:13	1500	56000	0.28	0.10
Lienden							
June 1973	129	1133	1:9	490	17000	0.26	0.07
June 1974	251	2379	1:9	160	55000	1.57	0.04
August 1974	203	4313	1:21	90	26000	2.26	0.17

Tabel 3. Vang- en loslaatgegevens van de proeven bij Overberg en Lienden. Alle getallen hebben betrekking op aantallen mannetjes in de gehele boomgaard.

to contribute to multiplication (Berlinger and Ankersmit, 1976). Moreover, there was an aftereffect of moths released by the end of August (Fig. 1).

The fraction captured (Table 3) was always higher for native moths than for reared moths. In June 1971 and 1974, their number was even higher than the estimated whole native male population. No figures are given for August 1973 as the pheromone traps during the peak of the flight failed to trap moths, probably through exhaustion of the bait. The capture ratio released: native 20:1, which should be used according to De Jong et al. (1971), was achieved only once. The ratios tended to be higher in August than in June. This was also true in August 1973 with a ratio of 23:1. Capture ratios were always extremely variable (Ankersmit, 1975). On 13 June 1973, we had a ratio of 90:1 (192 moths) and on 15 June only 2:1 (36 moths). Low capture ratios usually coincided with cool weather and low flight activity (Barel, 1973).

The number of native males caught in the light traps in the experimental orchard near Overberg increased 1.14 times from 61 per trap in the first flight to 70 in the second flight. In the chemically controlled orchard nearby, captures rose by 1.7 times from 191 to 325 per trap.

In the trial near Overberg, larval population increased tremendously from May to July but population level seemed reduced in May 1972 (Table 4). Then *A. orana* was rare in this orchard. In a nearby sprayed orchard of the same owner, it was more numerous in May 1971 than in the unsprayed experimental orchard (according to calculation). In July and September a similar infestation was found, while in May 1972 the difference was considerable through a large number of larvae on unsprayed trees of 'James Grieve' that had not been sprayed in previous August. Other tortricids increased in the experimental orchard but remained rare in the sprayed orchard. There, only one specimen of *Spilonota ocellana* was found.

Table 4. Results of larval counts at Overberg and Lienden.

Period	Number of trees examined	Number of larvae			Number *A. orana*/ha	
		A. orana	other tortricids	unknown	release orchard	sprayed orchard
Overberg						
May 1971	33	6	10	1	200	
July	3700 shoots	25	0	12	3000	3000
Sept.	25	12	86	108	500	600
25-26 April 1972	12	1	47	0	80	
16 May	25	2	72	0	80	1500
Lienden						
May 1973	61/5[1]	8	0	0	500	
July	375	395	33	9	830	
Sept.	90	152	88	0	1300	
May 1974	92	20	139	0	170	
July	57	7	91	20	100	
Sept.	75	63	786	18	660	
April-May 1975	85	4	644	19	40	

[1] Of 61 trees, per tree 1/5 part was examined.

Tabel 4. Resultaten van larvetellingen in Overberg en Lienden.

Table 5. Calculated numbers of leafrollers per ha during sampling in the orchard near Lienden.

	May '73	July '73	Sept. '73	May '74	July '74	Sept. '74	April/ May '75
Tortricinae:							
Acleris comariana } *A. sparsana*		19		300	1100	100	
Adoxophyes orana	500	830	1300	170	100	660	40
Archips podana		10		40	40	440	120
Pandemis ceresana		15		100	150	250	130
P. corylana			50				
P. heparana				150	30	40	590
Ptycholoma lecheana							160
Olethreutinae:							
Hedya nubiferana					30		50
Spilonota ocellana	65		710	620	30	7340	4830
Unknown		19	18		275	18	

Tabel 5. Berekend aantal bladrollers per ha bij de bemonstering van de boomgaard bij Lienden.

The trial near Lienden gave similar results (Table 4 and 5). After an initial increase in July of the first year, population declined and *A. orana* subsequently became rare. In Lienden too, other tortricids increased locally to high counts.

The distribution of larvae over the trees was extremely irregular. In September 1973, we found 68 of the total of 152 larvae on two trees and no larvae on 61 trees out of 90 trees. Statistical analysis of the many counts when only a few larvae were found was senseless. The results of counts when many larvae were found are given in Table 6. In two cases, there was a negative binomial distribution for the larvae over the trees: var (x) $= \bar{x} + \bar{x}^2/k$. The letter k is the exponent of negative binomial distribution. The value of k was always < 1.

In some cases, parasitism was determined (Table 7). Counts showed that parasitism was low in 1973 but increased in 1974. The parasite population changed. In July 1973, *Colpoclypeus forus* predominated; in July 1974 other species were prob-

Table 6. Statistical analysis of some counts.

July 1973	September 1973	September 1974
$\bar{x} = 1.05$	$\bar{x} = 1.69$	$\bar{x} = 0.87$
$s_x = 2.75$	$s_x = 5.41$	$s_x = 1.37$
$s_{\bar{x}} = 0.14$ (bin. distr.)	$s_{\bar{x}} = 0.57$ (bin. distr.)	$s_{\bar{x}} = 0.14$ (bin. distr.)
$k = 0.17$	$k = 0.15$	$k = 0.15$
$n = 375$	var (x) $= 20.73$ $s_{\bar{x}} = 0.48$	var (x) $= 2.354$
(No neg. binomial distribution established)	$n = 90$ (negative binomial)	$n = 75$ $s_{\bar{x}} = 0.18$ (negative binomial)

x = Number of larvae per tree; n = Number of trees examined; k = Exponent negative binomial.

Tabel 6. Statistische analyse van enkele tellingen.

Table 7. Total numbers of larvae of *A. orana* collected and fractions of parasitized larvae.

	July 1973	Sept. 1973	Sept. 1974	May 1975
Total number of *A. orana*	447	162	105	17
Fraction parasitized	0.12	0.06	0.4	0.76

Tabel 7. Totaal aantal verzamelde larven van A. orana en fracties geparasiteerde larven.

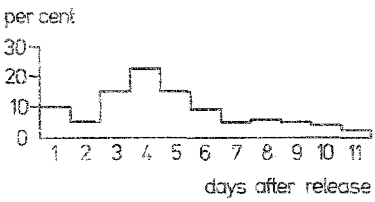

Fig. 1. Percentage of total recaptured of moths caught daily.

Fig. 1. Dagelijkse terugvangst van motten als percentage van de totale terugvangst.

ably more important. Out of 144 leafrollers with 18% parasitism, only 3 larvae were parasitized by *C. florus*. In September 1974 and in May 1975, *Diadegma praerogator* and *Apanteles xanthostigma* were the most important parasites of *A. orana*.

Recapture data (Fig. 1) revealed that about 20% of the released moths were still active one week after their release. In 1973, a peak in recapture was observed four days after release. In 1974, this peak was not found, perhaps because the traps were examined twice a week. A few moths may still be caught 18 days after release.

Discussion

The first question is: Did release of sterile males reduce the population? The gradual decline in numbers of *A. orana* according to larval counts in both orchards seems affirmative. Moreover, D. Snieder (Institute for Atomic Sciences in Agriculture, pers. commun.) found a few larvae with chromosomal aberrations in July and September 1973. This suggests matings between native females and sterilized males, as only 90–95% of these matings were expected to produce no offspring.

Counts were inaccurate and the variance could not always be determined. In September 1973, the ratio $S_{\bar{x}}/\bar{x}$ was 0.28 and in September 1974 was 0.21. However, the results of the counts seem not contradictory. They indicate a survival from September to the following May of 0.16 in 1971/72 and 0.13 in 1973/74. De Jong et al. (1971) mention a survival of 0.1 for this period among a high population. The counts of May 1975 indicated a higher mortality but then numbers were extremely low. Also the different rates of increase according to the light traps in the trial near Overberg support the idea of population suppression.

An objection to presumption of the effect of the sterile male method is the general observation that *A. orana* declines in numbers in neglected orchards (Gruys, 1975). Our experimental orchards resembled them in lack of chemical treatment. We can, however, consider that this reduction in numbers is not likely to occur during the first year after abandoning chemical control.

Parasitism is unlikely to be a major factor in the reduction of the *A. orana* population during the first year, but in the second year parasitism becomes important and it is then a major reducing factor (Table 7). Without parasitism, the population level of *A. orana* would have been almost the same as in May the year before. If so, the release effort would have compensated for multiplication and immigration.

A second question is: How important is immigration? According to calculations by H. G. Fransz (reported by De Jong et al., 1971) an immigration of 100 moths per ha per flight period can upset a release programme as the number released moths needed to keep a population low then increases tremendously.

The data in Table 3 suggest that immigration is likely in several flight periods as more native moths were caught than could be present according to our counts. This discrepancy in our data must be due either to underestimation of the population level or to immigration or to both.

Table 8 attempts to quantify immigration. We can assume that the ratio of recaptured reared moths to the total number of released moths will be nearly the same as the number of captured native moths to the total number of native moths. As flight activity for the reared moths is lower (Table 1 and 2), we must correct the total number of released moths. We used for this correction the factor $0.75 \times 0.8 = 0.6$ (Table 1 and 2). This permits calculation of the total number of native moths, which is given in the denominator of the expected ratio for native moths in Table 8. Subtraction from this number of the counted number gives the number of immigrated males. The results under the heading immigration show that usually a few hundred males immigrate. Only in June 1974 was immigration extremely large. As the native male population level was low, emigration could be neglected. The calculation method is rather insentive to errors in counting larvae in the field. An error in the recapture ratio of reared moths would have more effect. Such an error could arise when reared moths had an inherent lower capacity for flight and attraction to pheromone traps. Then another correction factor would have to be applied. Our longevity studies do not indicate any notable lower activity than we expected from native moths. Even when we use the higher ratio of June 1973 for June 1974, an immigration of about 2000 males can be calculated. From these results, we conclude that immigration will usually be too important for a successful eradication programme and that contin-

Table 8. Estimation of numbers of immigrated males after correction of data for irradiation and marking effects. All figures refer to males only.

Year	Flight period	Corrected ratio reared moths		Expected ratio native moths	Counted number native moths in whole orchard	Immigration (approx.)
1973	June	1133/10200	0.11	129/1170	490	700
	August	1166/22200	0.05	51/1020	800	200
1974	June	2379/33000	0.07	251/3586	160	3400
	August	4313/15600	0.28	203/725	90	600

Tabel 8. Schatting van het aantal geïmmigreerde mannetjes na correctie van de gegevens voor stralings- en merkinvloeden (alle getallen hebben alleen op mannetjes betrekking).

uous release of large numbers of sterile moths will be necessary to deal with immigrants.

The third question is: What happens to the numbers of other insect pests when chemical insect control is omitted? The results of counts in Table 4 and 5 indicate the sharp increase in many species of leafroller. In May 1973, only two species were found with *A. orana* dominant (Table 5). In May 1974, six species were found, among which *Spilonota ocellana* was most numerous. In May 1975, there were seven species with *S. ocellana* far outnumbering the others. This spectrum of species resembles that of neglected orchards. A similar phenomenon was found in Overberg (Ankersmit, 1975). These other tortricids were held largely responsible for a 4% damage to the fruits of Cox's Orange Pippin in 1973 and 9.4% damage in 1974. This increase also means that the goal of the sterile male method against *A. orana* cannot be reached completely as chemical control remains necessary.

The results of the counts in Table 5 indicate a multiplication factor for *S. ocellana* of 8 and for *Pandemis heparana* of 4 per generation. They also show the importance of counting at the correct moment. For *S. ocellana*, September is too late. It is most unlikely that the reduction in numbers from September to May can be so small as found for this species. *A. orana* always shows an important winter mortality. The period of July too is obviously unsuitable for counting *S. ocellana*.

The most likely explanation of the increase in other tortricids is omission of chemical control. These species have only one generation per year and multiply less quickly than *A. orana*. They are therefore easier to control. Chemical control of *A. orana* is applied according to a well developed warning system. Sprays at the wrong moment usually have poor results for this species. It is likely that this applies to other leafroller species that have a phenology somewhat different from *A. orana*. The insecticide sensitive first-instar larvae are not present at the same moment as those of *A. orana*, and consequently the sprays against *A. orana* may kill only few of them. Also preblossom sprays are only partially effective. The explanation is therefore doubtful.

Another possibility could be competition between larvae for space. *A. orana* is highly aggressive (Ankersmit and Van der Meer, 1973) and attacks intruders in its territory . Perhaps both reasons are valid, but their relative importance cannot be judged from our observations.

The results as a whole indicate an effect of the sterile male method. Even moderate immigration will upset large-scale release programmes. As other species of leafrollers increase, chemical control of Lepidoptera remains necessary and the method will not achieve its purpose. A specific control method will not therefore always be a solution to a pest problem in integrated control.

Samenvatting

Onderzoek over de steriele-mannetjestechniek als bestrijdingswijze van Adoxophyes orana (Lepidoptera, Tortricidae). 5. Loslaatproeven

Twee loslaatproeven met steriele mannetjes ter bestrijding van de vruchtbladroller *Adoxophyes orana* worden beschreven.

In de eerste, uitgevoerd in 1971 bij Overberg, werden 41 000, met 25 krad gamma-

stralen gesteriliseerde mannetjes bij de eerste vlucht, en 56000 tijdens de tweede vlucht losgelaten. In geen van de proeven werden de mannetjes van de wijfjes gescheiden. De sexe verhouding was circa 1:1. Alle losgelaten motten waren gemerkt met Calco Red Oil D. Terugvangsten met vanglampen gaven een verhouding tussen losgelaten en wilde motten van 4:1 in de eerste vlucht en 13:1 in de tweede vlucht (Tabel 3). Tellingen van de aantallen larven, door het afzoeken van hele bomen, wezen op teruggang van het aantal rupsen (Tabel 4). De vangst aan wilde mannetjes in de vanglamp wijst op een duidelijke immigratie uit de omgeving (Tabel 3). Het aantal andere bladrollersoorten bleek sterk toe te nemen (Tabel 4).

In de tweede proef, uitgevoerd in 1973 en 1974 bij Lienden, werden tijdens de eerste en tweede vlucht van 1973 resp. 17000 en 37000 mannetjes losgelaten en in 1974 resp. 55000 en 26000. De bestralingsdosis bedroeg 25 krad. De wijfjes werden in gelijke aantallen mee losgelaten. De motten waren gemerkt met 'Rotor kleurstoffen' en werden teruggevangen in feromoonvallen. De terugvangverhouding bedroeg in 1973 tijdens de eerste vlucht 9:1 (Tabel 3). In 1974 was deze resp. 9:1 en 21:1. De larvetellingen (Tabel 4) wezen weer op sterke teruggang van *A. orana*. Ook hier bleken andere soorten sterk in aantal toe te nemen (Tabel 5) zodat toch nog een vrij belangrijke bladrollerschade in de oogst ontstond.

Hoewel de populatiedaling in beide proeven zeer duidelijk was, kan toch nog niet met zekerheid tot succes van de methode worden geconcludeerd omdat in de laatste jaren de betekenis van *A. orana* in geïntegreerd bestreden percelen afnam. Een belangrijke moeilijkheid blijkt ook de statistische bewerking van de tellingen te zijn, daar *A. orana* zeer sterk pleksgewijs voorkomt. Het verdelingstype kon niet altijd worden vastgesteld maar leek soms op dat van een negatieve binomiaal met k-waarden kleiner dan 1 (Tabel 6).

Ook in deze proef werden aanwijzingen voor immigratie gevonden (Tabel 8). Deze is niet zo sterk dat het loslaatprogramma werd verstoord maar zou wel een uitvoerige campagne in de weg staan.

Door het toenemend optreden van andere bladrollersoorten wordt het doel van de specifieke bestrijding van *A. orana* verijdeld, daar toch nog chemische bestrijding noodzakelijk blijft. De specifieke bestrijdingsmethode hoeft dus niet altijd een oplossing van een plaagprobleem in de geïntegreerde bestrijding te geven.

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