

# Illustrated notes on the biology of *Sphinctus serotinus* Gravenhorst (Hymenoptera, Tryphoninae, Sphinctini)

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## Abstract

Field and experimental observations on the European ichneumonid *Sphinctus serotinus* as a koinobiont ectoparasitoid of the limacodid moth *Apoda limacodes* are recorded. The egg is anchored into the extremely thick cuticle of its host but not deeply enough so that it would survive host ecdysis. That may explain the late summer-early autumn flight time of the parasitoid, when practically all host larvae that remain are in their final instar, though avoiding competition from other parasitoids may also play a part. The tough cuticle of the host probably also underlies the lack of host feeding by the adult parasitoid. There is no ability to avoid superparasitism, though self-superparasitism is limited by post-oviposition flight to leave the host. The egg can be laid with its chorion still uncoloured when hosts are in plentiful supply, but such eggs become the usual pale brown colour within a short time. No dumping of eggs in the absence of hosts occurs. The egg normally doesn't hatch until after the host has prepared its cocoon, and the parasitoid larva, still anchored in the eggshell at first, feeds very slowly through the winter and early summer on the prepupal host. Development through the prepupal and pupal stages within the host cocoon similarly proceed slowly, with no evident diapause at any stage. The various stages of the life cycle are illustrated photographically.

## Keywords

Life history, biology, egg, larva, pupa, superparasitism, *Apoda limacodes*, Netherlands, Veluwe

## Introduction

Sphinctini is one of seven tribes recognised in the ichneumonid subfamily Tryphoninae (Bennett 2015) and contains 14 described species, distributed largely in the Palaearctic and Indo-oriental regions but also in the neotropics (Yu et al. 2012). According to Kasparyan (1992) all the temperate species known at that time fly in the late summer and early autumn. One species, *Sphinctus serotinus* Gravenhorst, occurs widely in Western Europe (Fauna Europaea 2015), and has long been recognised as a specialized parasitoid of the limacodid moth *Apoda limacodes* (Hufnagel). However, it is infrequently collected and it remains rather poorly known. Hinz (1976) reported in outline its oviposition behaviour and the unusual structure of the ovaries in the course of rearing a series in captivity on this host, in particular demonstrating that it is a koinobiont ectoparasitoid and securing its placement within Tryphoninae, but that paper was not illustrated and further aspects of the parasitoid's biology were not covered. The present account consists of both field observation in the Netherlands (JV) and experimentation (MRS), and gives some additional information and, particularly, some illustrative photographs.

## Materials and methods

The source of wild *Apoda limacodes* and *Sphinctus serotinus* used to establish cultures for experiments in Edinburgh was the Veluwe (Gelderland) area in the Netherlands, where collections and field observations were made by JV. Host cocoons, including some resulting from larvae bearing eggs of the parasitoid, were sent to MRS in xi.2005, and the following year a culture of the host was set up in readiness for the emergence of the parasitoid adults in autumn. Once in Edinburgh, livestock was kept under semi-natural conditions in a detached, shaded and copiously ventilated rearing shed (cf. Shaw 1997), except for the brief periods of experimentation and photography undertaken indoors. At all times *A. limacodes* larvae were fed in closed plastic boxes (variously 18 × 12 × 6 and 14 × 8 × 6 cm) on *Quercus robur* leaves and, rather than using tissue paper liners, the boxes were frequently opened and wiped out to prevent excessive condensation of moisture instead. This protocol is vital because of the apodous nature of the larvae, and their reliance on secreted slime for locomotion.

When an adult female of *S. serotinus* emerged on 12.ix.2006 it was fed ad libitum on honey:water (1:3) and remained unmated. After six days it was offered cultured final instar larvae (the only instar by then available) of *A. limacodes* singly and under continuous observation at various times over the period until 26.ix.2006 (14 days after emergence), after which no further hosts were available. Initially the host larva, on the *Quercus* leaf on which it had been feeding or resting, and the parasitoid were confined under a transparent plastic container but, as the parasitoid's interest in the host increased, the cover was removed to allow unimpeded observation and photography. A further female of *S. serotinus* hatched on 27.ix.2006 from the 2005 field collections;

its general behaviour was similar, but no hosts were by then available for oviposition. Some observations and photographs involving a field-caught female in the Netherlands are included in the account below.

Parasitized hosts were allowed to continue feeding until cocoon formation, and (both from the wild-collected 2005 and experimental 2006 material) cocoons were opened at intervals during the ensuing winter and subsequent summer to follow the progress of the parasitoid within. As is well known (e. g. Porter 1997), *Apoda limacodes* passes the winter as a strongly cocooned prepupa and pupates in the spring.

One wild-collected host bearing an egg was fixed in 4% formaldehyde (ca 30 hr) and the relevant portion dehydrated with ethanol (50% to 96% in 5 stages) then degreased with 4 changes of xylol before being embedded and sectioned in paraffin wax. Sections (8 microns) were stained with Haematoxylin and Eosin, then slide-mounted in Malinol, in order to investigate details of the egg's attachment.

Photographic images were obtained digitally on a Konica Minolta Dynax 7D camera with Minolta 1-3 macro zoom lens and a ring flash, or on 35 mm Fuji colour transparency film using a standard SLR camera body and a Medical Nikkor 120 mm lens with automatic ring flash.

## Results

### Field observations

In the Veluwe, larvae of *A. limacodes* have, at least in recent years, occurred commonly on *Quercus robur* growing under a wide range of conditions, from quite dense woodland (where it is also common on *Fagus sylvatica*) to open heathland (Fig. 1), with 2004, 2005, 2008 and 2014 being years of particularly high abundance. Larvae, which exhibit some colour polymorphism not connected with parasitism (Figs 2, 3; final instar), were present in the field for a long period, from late May until early October, in variable stages of growth. By mid-September many *A. limacodes* larvae had become fully grown and spun their cocoons, and virtually all remaining were in their final instars. Attack from other parasitoids (in the area the campoplegine ichneumonid *Phobocampe alticollis* (Thomson), the microgastrine braconid *Dolichogenidea lacteicolor* (Viereck), and the rogadine braconid *Triraphis tricolor* (Wesmael)) was by then finished, so the *A. limacodes* larvae that remained were unparasitized. From late September onwards host larvae were increasingly found bearing eggs of *Sphinctus serotinus* (the first one seen on 25.ix.2005), but almost exclusively on relatively isolated *Q. robur* trees in open sunny heathland situations — in particular at Edese Heide (Fig. 1) where, as late September and the first half of October wore on, the majority of *A. limacodes* larvae were found to bear one or more eggs (Figs 2, 3) of this strictly solitary parasitoid. Good numbers of the host occurred also in more shaded woodland in the Veluwe, on both *Q. robur* and *F. sylvatica*, but the parasitoid was essentially absent from this situation and none were found on hosts feeding on the latter tree (which was lacking in the open, xerothermic, areas favoured by the parasitoid).



**Figure 1.** Biotope at Edese Heide with *Quercus robur*.



**Figures 2, 3.** Final instar larva of *Apoda limacodes* with **2** one egg **3** four eggs of *Sphinctus serotinus*.

In the wild eggs of *S. serotinus* were seen on a large number of hosts, and appeared to be positioned more or less at random on the exposed surface of the host's body, including positions well down at the sides and near both anterior and posterior ends, though more central and dorsal positions were commonest. Many instances of two, three and even four eggs on a single host were seen (Fig. 3).

### Behaviour and captive rearing

The adult female is about 14 mm long and adopts a fully-resting stance (i. e. when asleep; Figs 4, 5) that is rather unusual in Ichneumonidae, with wings spread away

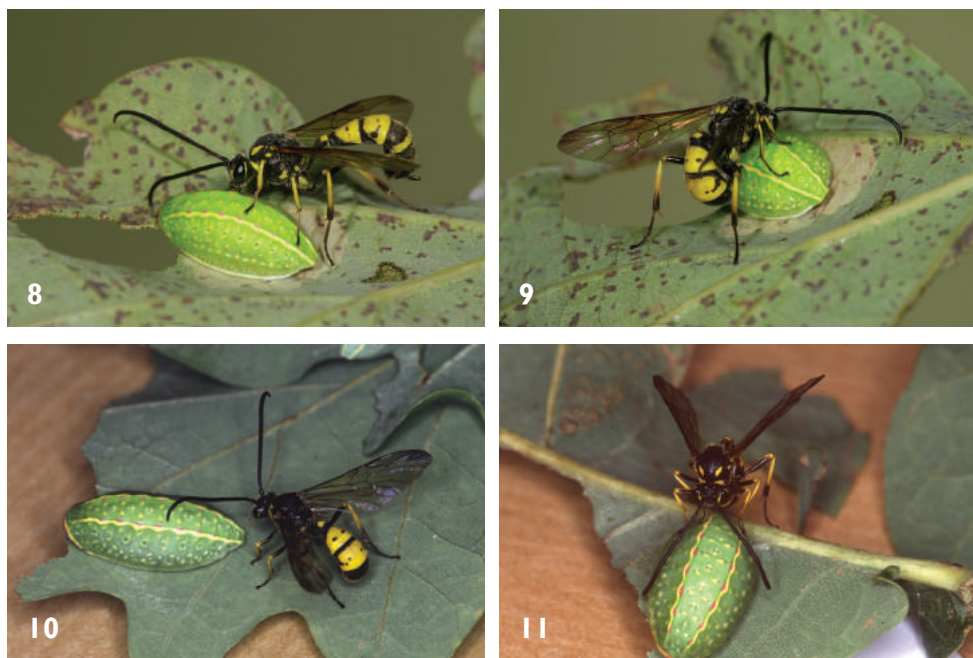




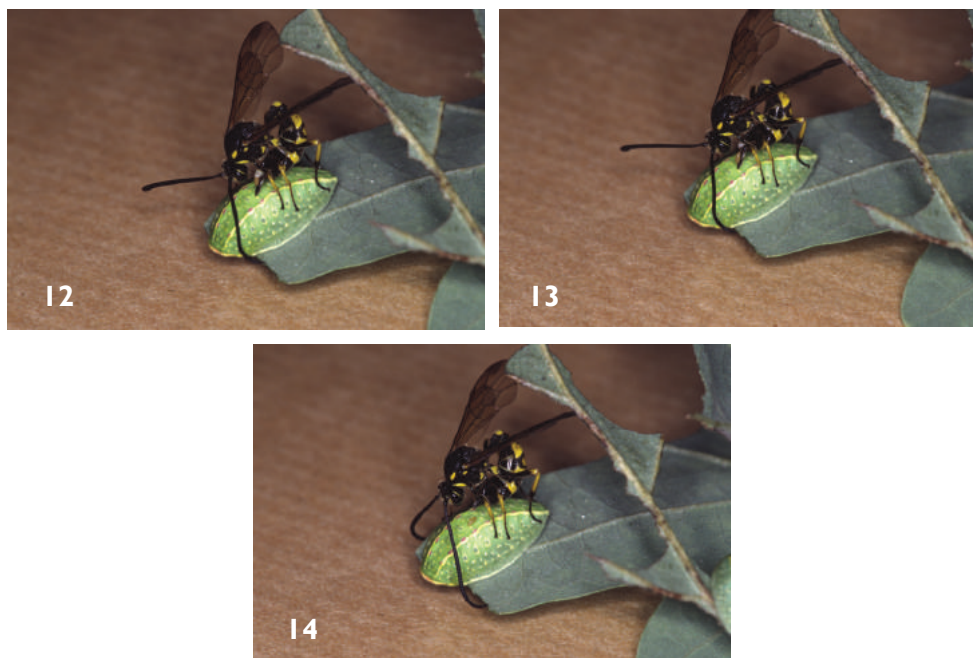
**Figures 4–7.** *Sphinctus serotinus*, 4, 5 asleep 6, 7 in motion.

from the metasoma, but which clearly accentuates its mimicry of vespid wasps (perhaps *Eumenes* in particular). This exposure of the metasoma is maintained while the parasitoid is actively searching on foot (Figs 6–8), though at such times the antennae are directed forwards and sideways and the wing tips are not flattened to the substrate. The experimental female fed avidly on dilute honey, but showed no tendency to host feed. Even when deprived of hosts, eggs were neither dumped nor carried externally at the apex of the ovipositor.

The female was first offered hosts six days after emergence, when she started to show weak interest in host traces (chewed leaf edges, and the slime trails by which the host moves). This interest became stronger on subsequent days. When a host was encountered (Figs 8, 9) it was often antennated with one antenna along each side of the host's long axis, which often (not always) caused the host to rock rhythmically from side to side (a behaviour that is provoked by various kinds of disturbance, including from another *A. limacodes* larva, and did not unduly delay the female parasitoid although a slight deterrent effect was evident). The position of the antennae suggested that the host was being measured: all hosts offered were final instar, but it was clear from all interactions and ovipositions that large (14 mm or more, female) hosts were much preferred to smaller ones (9–12 mm, male), though some ovipositions on the smaller size range did occur. Except that the antennae were curled downwards during actual oviposition and in the subsequent brief period of inspection (but usually not in contact with anything), they were not used for further investigation or manipula-



**Figures 8–11.** *Sphinctus serotinus*, approaching and accepting *Apoda limacodes* larva.



**Figures 12–14.** *Sphinctus serotinus*, oviposition sequence.



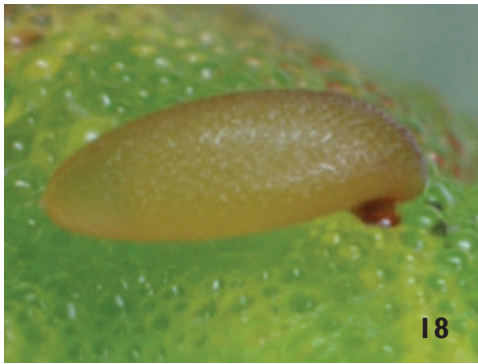
**Figure 15.** *Sphinctus serotinus*, priming for oviposition.

tion. Altogether 15 ovipositions were obtained, interspersed with rejections that were sometimes later reversed, on days 6 (2 ovipositions), 8 (1), 9 (1), 13 (7) and 14 (4) of this single female's adult life. When the female crawled, often rather slowly, onto the host (Fig. 10) she sometimes did so transversely and kept the hind legs on the substrate (Fig. 11), but usually she approached longitudinally and then used all six legs to perch upon it (Fig. 12). This difference accounts for the variable egg positions seen (respectively lateral and dorsal). The ovipositor was then brought forward between her legs to pierce the host integument, with the sheaths supporting the ovipositor. The main body of the egg issued from the genital opening caudal end first (Fig. 12) and slid down the outside of the ovipositor, with its anchor travelling down the shaft internally (Fig. 13) until oviposition was completed, leaving the egg on the surface of the host but its stalk implanted (Fig. 14). This process took about 10–15 (exceptionally 20) seconds, during which the host was usually completely quiescent, but appeared not to have suffered temporary paralysis. The female usually paused, apparently reviewing her achievement, for a few seconds after oviposition then, invariably, left purposefully — usually by flying away. A host already bearing an egg was accepted without any sign of hesitation or inhibition, so this prompt post-oviposition reflex departure seems to be the only way in which self-superparasitism is avoided. Normally the female fed avidly on honey:water between ovipositions, and there was sometimes a clear cocking and jerking motion at the apex of the metasoma suggestive of a new egg being moved down the oviduct to a position in the genital opening ready for oviposition as a host was approached (Fig. 15). The anchored egg is usually light brown at the time of oviposition





**Figures 16–17.** *Sphinctus serotinus*, laying an immature egg.



**Figures 18–20.** *Sphinctus serotinus*, egg on host 18 as laid 19, 20 sectioned.

and remains so until it hatches, but if more than two ovipositions occurred in close succession the subsequent eggs were very pale yellowish green (Figs 16, 17), darkening to the usual light brown over a few hours. After the available hosts had run out the female was denied access to food, and died (undoubtedly of starvation) nine days later.

The matt, rather roughened egg of *S. serotinus* is 1.1 mm long and anchored into the host integument (Figs 18–20), as is typical for Tryphoninae (Bennett 2015). In this case the egg is fastened by a clear flange-like expansion at the distal end of its stalk. The anchor extends only into the already secreted cuticle which is abnormally thick in *Apoda*





**Figure 21.** *Sphinctus serotinus*, egg chorion splitting.



**Figures 22–23.** *Sphinctus serotinus*, first instar larva feeding.

(stained pink in Figs 19 and 20), and not through it into the deeper cell layers involved in secretion of new cuticle. The stalk of the egg appears to stain in a different way from the other collagens involved in the structure of the egg, but the stains used do not adequately characterise it (C. Gielis, pers. comm.). Normally the egg of *S. serotinus* does not hatch until the host has made its cocoon, but under artificially high humidity (closed plastic box) it may do so. The chorion splits (Fig. 21) at the anterior end (furthest from the anchor) but the setose first instar larva remains partly (caudally) in the eggshell as it starts to feed in the late autumn to early winter, as evidenced by a blackened feeding lesion (Figs 22, 23), with presumed urate patches already evident. The larva remains in this position, feeding slowly on the cocooned host prepupa, through the winter but by the second week of May a larva in a cocoon opened for observation had left the eggshell behind, grown considerably and moulted at least once, now moving freely across the body of the still living host prepupa and making new feeding lesions. The parasitoid larva was still setose and now very liberally dotted with presumed urate deposits. (In the milder early spring of 2007 the parasitoid larva in a cocoon opened on 19 March

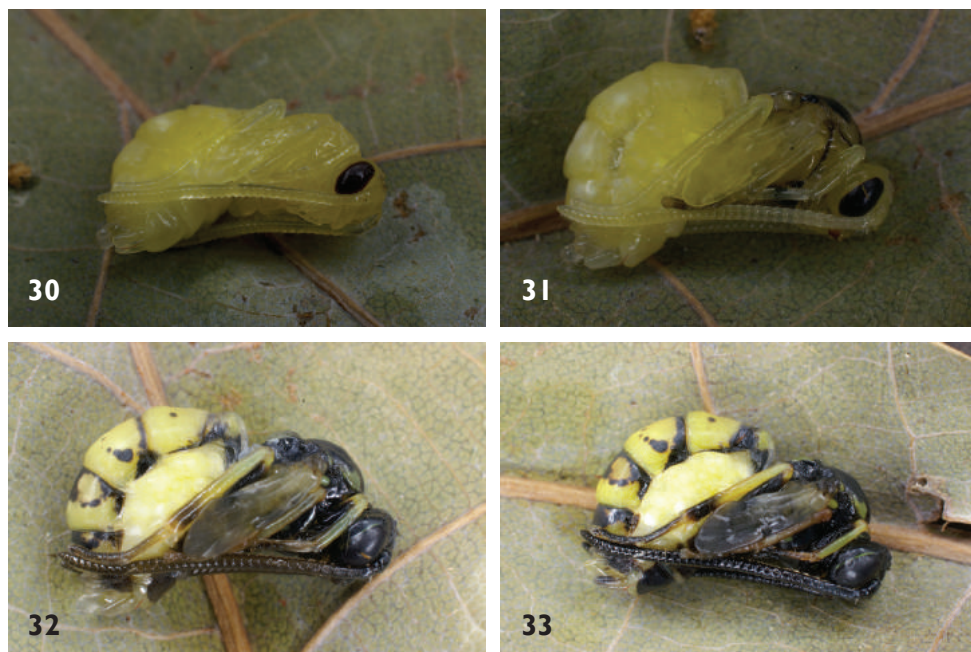


**Figures 24, 25.** *Sphinctus serotinus*, intermediate instars **25** showing exuviae.



**Figures 26–29.** *Sphinctus serotinus*, prepupa **26, 27** in situ **27, 29** removed from host cocoon.

in the Netherlands had already reached this state (Fig. 24).) It is unclear at what point the supernumeraries (in cases of superparasitism) are eliminated, or how, but presumably this cannot take place while the larvae are still confined by their eggshells. Growth continued slowly through the spring and early part of summer (in Edinburgh in 2006 the weather was particularly cold, with daytime temperatures in the rearing shed seldom exceeding 10 °C during May). The larva had moulted at least twice, probably three or possibly even four times, leaving the rather robust cast skins split along a dorsal line (Fig. 25), before dying about half grown around 11.vi.2006 — probably largely a result of excessive disturbance, but no doubt exacerbated by cold weather.



**Figures 30–33.** *Sphinctus serotinus*, pupa **30** fresh **31–33** progressively developing adult colour.

A further host cocoon (ca 8.5 mm long) containing *S. serotinus* was opened on 25.vii.2006, and by this date the parasitoid had completed its feeding and was prepupal. (The Edinburgh weather had been much warmer during late June and July, with temperatures regularly in the low 20s°C.) The host cocoon now contained an area of coarse white silk, at its midlength, isolating the host remains between the host cocoon wall and a frail white diaphanous cocoon enclosing the *S. serotinus* prepupa (Figs 26–28). The isolating wall of silk was positioned against the venter of the parasitoid's cocoon, within which faeces were visible. The *Sphinctus* cocoon was easily removed (Fig. 28) and opened, but the prepupa (whose eye spots were clearly evident) could not be withdrawn without damage because it was anchored firmly, via its anus, to its faecal material by a tough viscous/elastic strand of brown material capable of supporting much more than its weight (Fig. 29). This individual was kept under observation and pupated on 1.viii.2006. Unlike the prepupa, the fresh pupa is easily removed from its cocoon (Fig. 30). One pupa was brought indoors on 25.viii.2006 after which it gradually developed colour (Figs 31–33), the mesoscutum being the first area to darken and the legs among the last.

On one host that had received an egg the parasitoid failed to develop beyond its first instar, and the host then progressed to the (pharate) adult stage, indicating that no venom component that interferes with the host's endocrine system had been injected to control host development.

## Discussion

Despite its wide distribution (cf. Fauna Europaea 2015) *Sphinctus serotinus* is regarded as a rare insect in Europe, and indeed for several countries with historical records there are no recent reports: for example, the 19<sup>th</sup> century records for England given by Morley (1911) have not been repeated. However, despite its conspicuous colouring, it may be easily overlooked. The early autumnal flight time, appearance and behaviour of the adult would cause it to be easily passed over as a vespid, and perhaps given a wide berth even by entomologists at a time of year when stings from the numerous drowsy vespid workers are most likely to occur. Also, it seems not to be prominent in the field even where it is abundant: in the part of the Netherlands where it is so easy to find it in the egg stage, JV has seen only one adult during many hours of collecting its host, and indeed its eggs, during its flight time. Thus, like many parasitoids, its presence or absence may be much easier to investigate by finding its host than by depending on encountering adults, and even in Britain it may not be appropriate to regard it as extinct until a thorough search on that basis is made.

It was unexpected that the larval development was so gradual, and that there was no evident period of diapause, although the pupa was certainly rather slow to commence the process of adult cuticular development. Probably the timing of events was unduly affected by the cold Edinburgh climate (certainly a good deal colder than the Veluwe, or the areas of southern England where the host, and indeed *S. serotinus* in former times, have been recorded) but, significantly, there was no prolonged intact egg or pre-emergence adult stage, and the prepupal stage was reached only well into the summer.

The only contact assessment of the host that the female makes with her rather robust and scarcely flexing antennae appears to be in relation to host size, and the antennae seemed to be of low importance in tracking the host via leaf-surface traces and recognising it once found. However, any possible involvement of the antennae in courtship behaviour could not be investigated.

The egg evidently can be laid even when its chorion is sub-mature, and as many as seven were laid by the experimental female in one day. Although feeding on honey:water was quite ravenous on that day (between ovipositions) there was no tendency to host-feed — perhaps surprisingly in view of the host-feeding seen in other tryphonine parasitoids of Lepidoptera such as *Netelia*, in which the mandibles are used to make the necessary wound (Shaw 2001), until the very tough nature of the integument of *Apoda* larvae is considered. Host-feeding for *S. serotinus* may be simply too lengthy and risky a business to be viable. The anchored egg is embedded only in the host's outer cuticle, suggesting that only final instar hosts would be suitable for the parasitoid's development, as *S. serotinus* does not inject a development-changing venom and the egg would be easily sloughed off along with host cuticle at ecdysis. This is unlike the situation seen in the phytodietine tryphonine genus *Netelia* in which there is also no venom effect controlling host development (Shaw 2001). When a penultimate instar host is parasitized by *Netelia*, survival of host ecdysis by the egg tearing through the cuticle



being sloughed is readily achieved (Shaw 2001). The tough and thick cuticle of *Apoda limacodes* arguably limits the integumental tissues into which the egg can be anchored which may explain, at least as much as the lack of competition from other parasitoids, why *S. serotinus* has such a late flight time—in early autumn, when essentially all encountered host larvae will be in the final instar. This scenario also seems likely for other temperate *Sphinctus* species with similar limacodid hosts.

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