

## Seasonal Characteristics of Wood Formation in the Elite Genetic – Based *Eucalyptus camaldulensis* Dehnh.

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### ABSTRACT

Seasonal characteristics of wood formation were investigated using the knife-cutting method in five superior clones of six years old *Eucalyptus camaldulensis* in the plantation of eastern Thailand. In the observation of wound tissue formed through knife insertion, cambial initials at the time of knife – cutting could be estimated. Based on these positions, pursuit of time course of wood formation or radial growth was carried out.

In rainy season all clones showed active radial growth. The radial growth rate decreased in dry season. In the comparison of radial growth among five clones, clones 3 and 4 maintained better growth rate than other clones even in dry season.

**Key words:** *Eucalyptus camaldulensis*, wood formation, radial growth, elite genetic-based, seasonal characteristics

### INTRODUCTION

In the tropical monsoon area such as Thailand with long dry season, *Eucalyptus camaldulensis*, the fast growing species which can be adaptable to many climate condition (Kauman *et al.*, 1995), particularly, in the dry habitat area has been selected as one of the potential plantation species. Seedling or young plants for plantation are generally obtained from seeds or cuttings. For producing much amount of young plants, the group of Faculty of Forestry, Kasetsart University started to produce young plants through tissue culture technology in 1989. They could succeed to get young plants from tissue culture, succeeded in naturalization and planted them in field in 1992. The growth of each plant in the field of one-year-

old were varied distinctively. The trees from superior clones showed better growth and more uniformity than the trees from seedling (Siripatanadilok *et al.*, 1992).

The one important target in *E. camaldulensis* plantation from tissue culture is to evaluate the seasonal characteristics of wood formation or radial growth of each clone. Especially, the selection of clones with good growth rate in dry season is the key research point.

From the view point of the methodology to investigate the seasonal characteristics of wood formation or radial increment, the pinning method (Wolter, 1968) is excellent method because it is simpler and easier, marks accurately and can be adapted to a range of small to large diameter trees (Nobuchi *et al.*, 1993). In this method cambial

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initials at the time of pinning are recorded in the wound tissue (Nobuchi *et al.*, 1995). This method mostly has been applied to the study of temperate zone trees (Shimaji and Nagatsuka, 1971; Kuroda and Shimaji, 1983, 1984) and rarely applied to tropical trees.

For the pinning method to tropical trees, Shiokura (1989) and Nobuchi *et al.* (1995, 1996) used a nail instead of a pin because tropical trees have generally thick and hard bark. The wound tissues induced by either a pin or a nail insertion were short in longitudinal direction, therefore, it was difficult to obtain appropriate sections from the center of tiny wound tissues. To make this method easier "knife-cutting method" proposed by Fujiwara (1992) was the alternative method to improve the pinning method. Kuroda and Kiyono (1997) investigated the seasonal rhythms of xylem growth measured by knife-cutting method that they called the wounding method.

The researches of the seasonal characteristics of wood formation of genus *Eucalyptus* are very much limited. Ogata *et al.* (1996) analysed the seasonality of wood formation in *E. tereticornis* and *E. saligna* by knife-cutting method and Kondo *et al.* (1996) analysed the radial growth in *E. dalrympleana* by knife-cutting method. Particularly, the radial growth compared among clones of *E. camaldulensis* are not reported.

In this report, the knife-cutting method was applied to compare the radial growth among

5 superior clones of *E. camaldulensis* and the data were discussed in correlation with the climatic data near the plantation site. The objective of this study aims to screen for the eucalyptus clone which is fast growing even in dry condition.

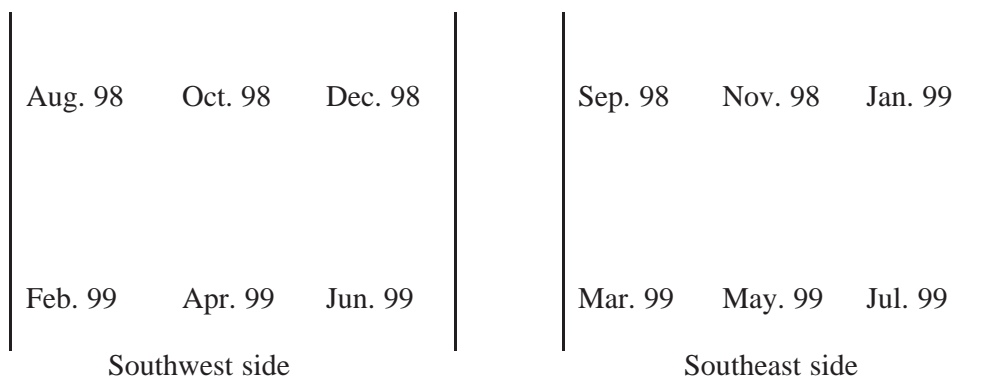
## MATERIALS AND METHODS

Five superior clones of six-year-old *E. camaldulensis* in the plantation site at Sakaew province in eastern part of Thailand were selected as sample trees. The name of clones were T5, Kitt, S9, Y2 and K2, which were coded in this report as clone 1, 2, 3, 4 and 5, respectively. Diameter at breast height ranged from 18.5 to 24.1 cm.

### Knife-cutting method

Knives of 0.5 mm in thickness and 10 mm in width were used for marking. To investigate radial growth around the trunk, marking positions were done at four sides of trunk (southwest side and opposite, southeast side and opposite). The knife was inserted deep enough to reach the border of cambium and xylem and removed immediately. As shown in Figure 1, the distance between two points was about 4 cm in tangential direction and about 10 cm in longitudinal direction. The marking was done every month from August 1998 to July 1999. Table 1 indicates the dates of marking.

Blocks of 3 cm in radial depth containing



**Figure 1** Schematic illustration of the marking point on a trunk.

the wounding tissues were collected on August 28, 1999 by a saw and a chisel without felling the trees.

### Light microscopy

Wood blocks containing the marked parts were incised through the center of long slit-like wound tissue by handsaw. Thereby, the wood blocks were separated into two parts. After first part of wood blocks were softened in boiling water, transverse sections 20-30  $\mu\text{m}$  in thickness were cut using a sliding microtome. They were stained with safranin fast green. Wound tissues were observed under a light microscope. The other part of the wood block was also used for the measurement of radial growth.

### Measurements of radial growth

Second part of wood blocks containing the wound tissues were sanded with a series of sand papers of No.40, 60, 80 and 120. Each of the sanded transverse sections were scanned using Epson ES-8000 image scanner and Adobe Photoshop program. For the measurement of radial growth in the computer, a growth ring boundary was used as a reference line. That is, radial growths were measured by computer as the distance between growth ring boundary and estimated cambial initials at the time of each marking.

## RESULTS AND DISCUSSION

### Characteristics of growth rings

The characteristics of growth ring

boundary as a reference line were investigated in transverse sections and scanned pictures. The concentric line was necessary for the measurement of radial growth of different marking positions around the circumference of the trunk.

As shown in Figure 2, the growth ring boundaries of all clones were not so distinct as those of temperate zone trees. It was because vessel diameters showed little variation throughout one year. However, late wood-like zone had slightly smaller diameter of vessels together with thicker fiber cell walls. Based on those characteristics, the growth ring boundary was adopted as a reference line.

### Anatomical characteristics of wound tissue

A transverse section of wound tissue induced by knife-cutting is shown in Figure 3. Nobuchi *et al.* (1995) and Ogata *et al.* (1996) reported that the wound tissue was divided into two zones. In zone 1 in Figure 4, cells in cambial initials and enlarging zone were directly affected by knife-cutting. They were crushed and cell wall formation were interrupted in the cells living at the time of marking. Therefore, they retained the cell wall organization, even if they were deformed. In zone 2, cells in cambial initials and enlarging zone were not crushed but indirectly affected by knife-cutting resulting in the formation of abnormal cells. This zone showed a region of normal wood (NW), callus-like cells (C), radially flat cells (FC) and a layer with small diameter of vessels (S).

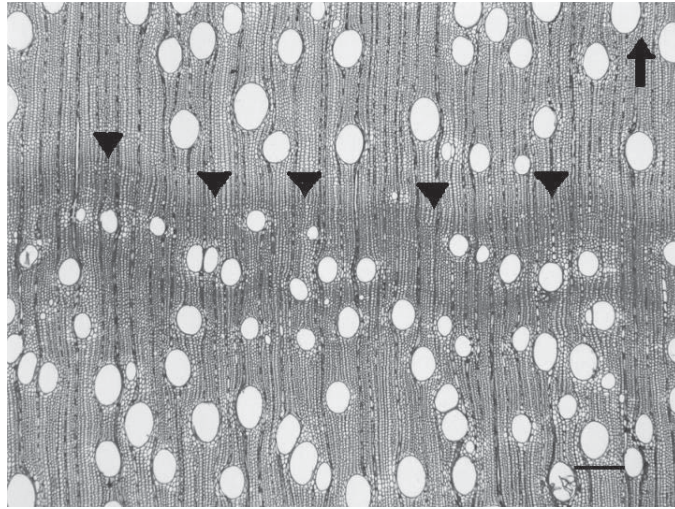
In the region with small diameter of

**Table 1** Dates of marking.

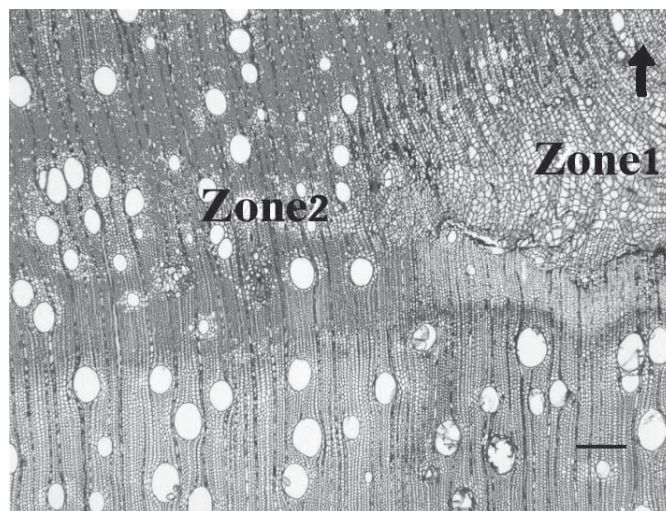
| Southwest side and opposite side | Southeast side and opposite side |
|----------------------------------|----------------------------------|
| August 12, 1998                  | September 12, 1998               |
| October 23, 1998                 | November 21, 1998                |
| December 26, 1998                | January 23, 1999                 |
| February 21, 1999                | March 17, 1999                   |
| April 27, 1999                   | May 22, 1999                     |
| June 20, 1999                    | July 17, 1999                    |

vessels a point where the number of cell rows increased tangentially or the group of radially flat cells was noted. This increase could have been caused by the anticlinal division of cambial initials. The line which connected the innermost point of anticlinal divisions (arrowheads) was considered theoretically to be the location of cambial initials

at the time of marking. Practically, this line coincided with the line of the innermost small-size vessels. This line was, therefore, adopted as the marker of cambial initials at the time of marking and used for measurement (Nobuchi *et al.*, 1995)



**Figure 2** A transverse section of clone 2 showing growth ring boundary or reference line. Arrowheads represent the reference line. Arrows in Fig. 2-5 indicate the direction of bark side. Scale bars = 250  $\mu\text{m}$ .



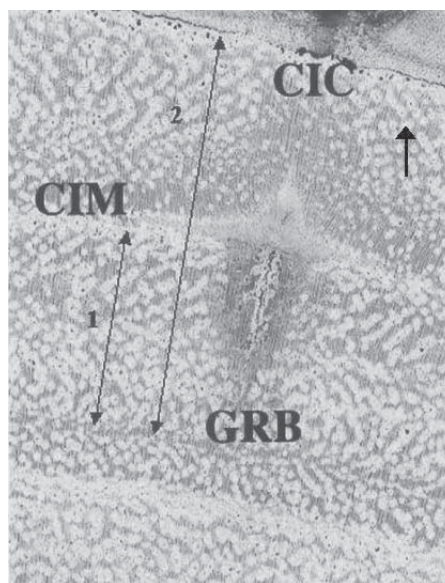
**Figure 3** A transverse section of clone 2 showing wound tissue induced by knife-cutting. The wound tissue was divided into two zones. Scale bars = 250  $\mu\text{m}$ .



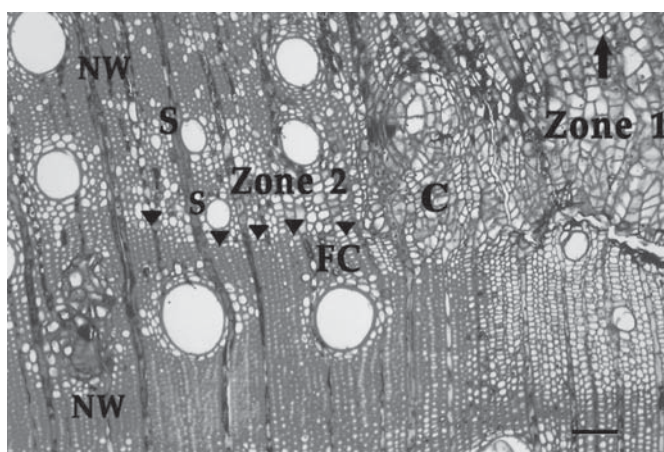
### Seasonality of radial growth and its relation to monthly rainfall

The distance between the reference line and the estimated cambial initials at the time of each marking was measured (line 1 in Figure 5). This indicates the radial growth in the terms from the latest growth ring boundary to the time of a given marking. However, It is quite natural that some errors might arise because the radial growth is not homogenous along the circumference of the trunk. To avoid these errors, the radial growth was expressed as the percentage of line 1 divided by line 2 in Figure 5 (Ogata *et al.*, 1996).

The percentage of radial growth rate compared among 5 clones are shown in Table 2. The radial growth rate in southwest side and southeast side of the trunks were the great dispersions. Shimaji and Nagatsuka (1971) reported that the greater dispersions of data might be attributed to the greater tangential width of pinning area. To eliminate such dispersions of data, the pinning area should be as narrow as possible. The dispersion of data did not overrule the fact that each growth curve was accurate growth pattern of the limited locus of each tree stem. Therefore, the growth curve was separated into two as shown in Figure 5 (a, b).



**Figure 5** A scanned transverse section of clone 3 showing the measurement procedure of growth rate. Line 1 shows the distance from the growth ring boundary (GRB) to the estimated cambial initials at the time of marking (CIM) and line 2 shows the distance from GRB to the cambial initials at the time of collecting wood block (CIC). Radial growth rate was calculated as the percentage of line 1 divided by line 2.



**Figure 4** A transverse section of clone 2 showing the enlargement of zone 2. Arrowheads show the estimated cambial initials at the time of marking. Normal wood (NW), callus-like cells (C), radially flat cells (FC) and small diameter of vessels (S). Scale bars = 100 mm.

**Table 2** Growth rate (%) of each marking in southwest side (SW) and southeast side (SE) of the trunks in 5 clones and monthly rainfall.

| Year and Month |      | Clone 1       |      | Clone 2       |      | Clone 3       |      | Clone 4       |      | Clone 5       |      | Monthly rainfall (mm) |
|----------------|------|---------------|------|---------------|------|---------------|------|---------------|------|---------------|------|-----------------------|
|                |      | Radial growth |      | Radial growth |      | Radial growth |      | Radial growth |      | Radial growth |      |                       |
|                |      | rate (%)      |      | rate (%)      |      | rate (%)      |      | rate (%)      |      | rate (%)      |      |                       |
|                |      | SW            | SE   | SW            | SE   | SW            | SE   | SW            | SE   | SW            | SE   |                       |
| 1998           | Aug. | 18.7          |      | 33.0          |      | 27.5          |      | 39.4          |      | 32.1          |      | 153.0                 |
|                | Sep. |               | 39.2 |               | 32.5 |               | 36.6 |               | 53.9 |               | 46.8 | 233.6                 |
|                | Oct. | 26.7          |      | 44.9          |      | 42.2          |      | 41.8          |      | 37.4          |      | 202.1                 |
|                | Nov. |               | 46.1 |               | 51.1 |               | 44.4 |               | 64.9 |               | 62.7 | 8.1                   |
|                | Dec. | 36.8          |      | 41.6          |      | 48.1          |      | 41.1          |      | 49.1          |      | 0.0                   |
| 1999           | Jan. |               | 63.0 |               | 59.3 |               | 59.4 |               | 67.0 |               | 70.5 | 15.0                  |
|                | Feb. | 37.8          |      | 52.8          |      | 56.7          |      | 53.2          |      | 51.3          |      | 0.0                   |
|                | Mar. |               | 49.3 |               | 52.2 |               | 63.1 |               | 67.4 |               | 66.5 | 122.0                 |
|                | Apr. | 47.0          |      | 60.7          |      | 59.8          |      | 50.5          |      | 50.0          |      | 78.9                  |
|                | May. |               | 72.2 |               | 77.4 |               | 80.0 |               | 77.1 |               | 80.1 | 129.0                 |
|                | Jun. | 66.9          |      | 81.6          |      | 82.6          |      | 80.7          |      | 79.4          |      | 306.0                 |
|                | Jul. |               | 87.2 |               | 86.6 |               | 92.5 |               | 86.9 |               | 89.9 | 83.4                  |

Figure 5 shows a transverse section with a wound tissue formed through knife insertion. Line 1 indicates the distance between the growth ring boundary (GRB) and the estimated cambial initials at the time of marking (CIM). Line 2 shows the distance between GRB and the cambial initials at the time of collecting wood block (CIC). Radial growth rate was expressed as the percentage of line 1 divided by line 2.

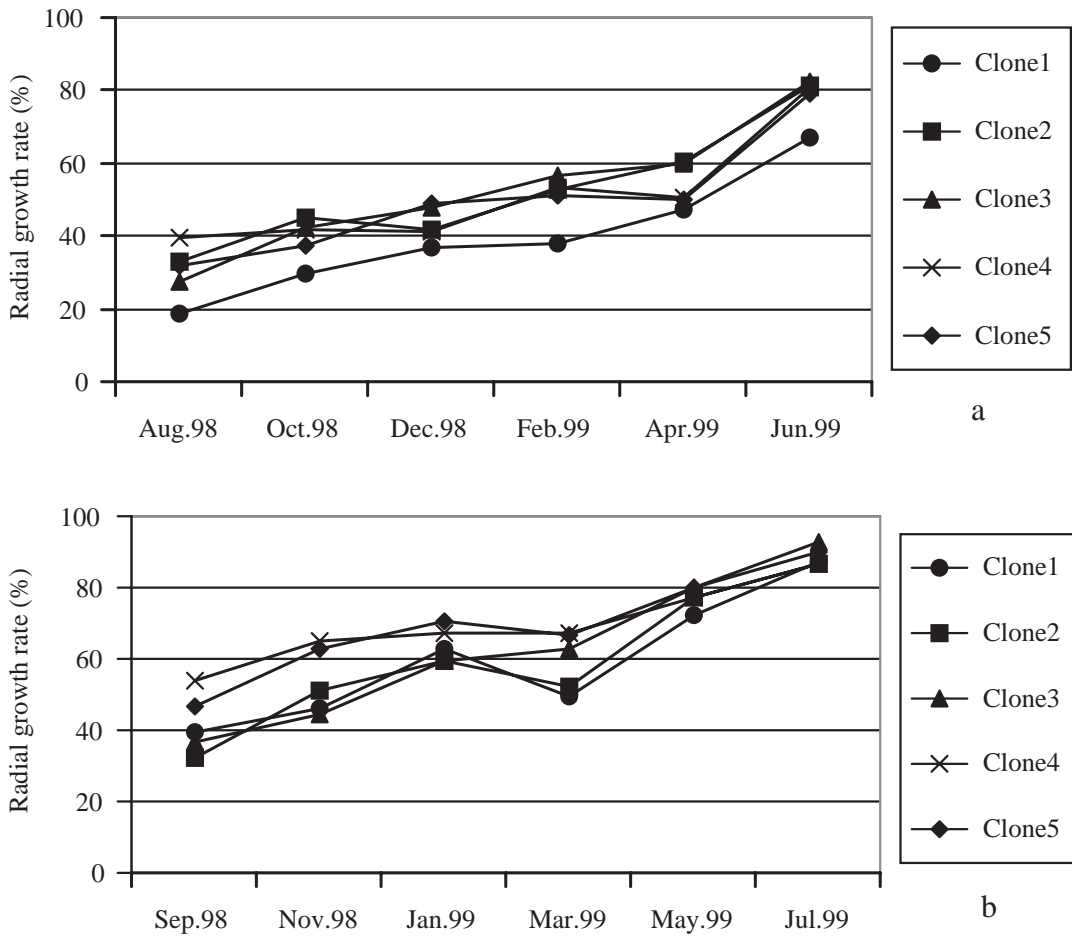
Data of radial growth rate (%) together with monthly rainfall are indicated in Table 2. The data of radial growth rate are also shown in Figure 6 (a, b) and corresponding monthly rainfalls are shown in Figure 7.

In Figure 6, 7 and Table 2, radial growth was judged to be active in rainy season and radial growth rate decreased in dry season for all 5 clones. This result was in coincidence with Nobuchi *et al.* (1995, 1996) who investigated seasonal characteristics of wood formation in *Hopea odorata*, *Shorea henryana* and *Tectona grandis* in Thailand.

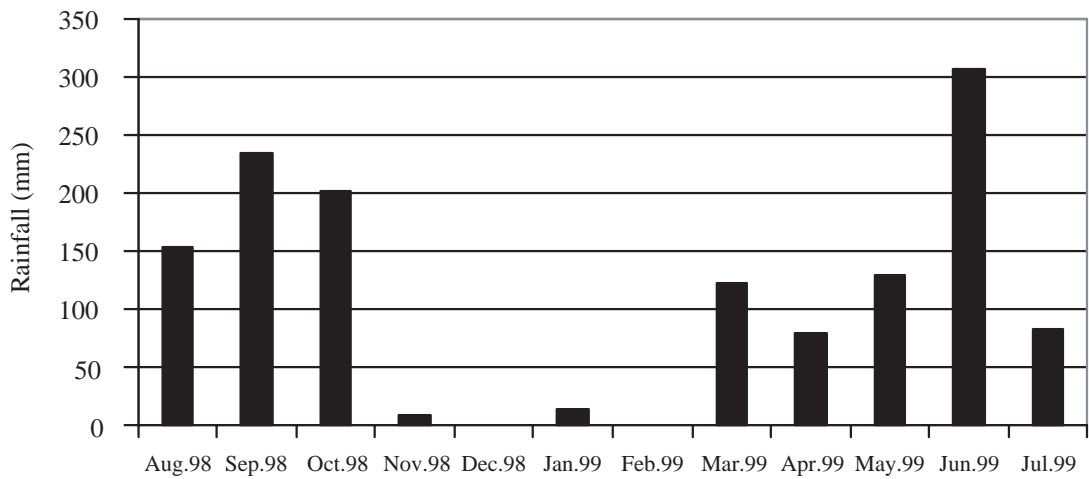
In the former part of dry season, wood

formation was considered not to stop even though the growth rate decreased. Considering that *E. camaldulensis* is the evergreen tree species, it is well understood. In the latter half of dry season, radial growth rate showed conspicuous decrease. We could not identify whether the wood formation completely stopped or not. For this investigation the observation of cambial pattern is necessary. Seasonal changes of soil moisture content are also important research point to discuss the necessary water supply to the cambial activities.

For the comparison among 5 clones, the radial growth patterns especially in the latter half of dry season were investigated. Some clones including clones 1, 2 and 5 showed more conspicuous decrease of growth rate. Clones 3 and 4 showed better growth rate. Especially clone 3 showed continuous radial growth even at the end of dry season which the soil moisture content would be very low. This result supported the idea that clone 3 would be more tolerant in dry habitat. When we consider the selection of *E. camaldulensis* clones, this is important information.



**Figure 6** Comparison of the growth curves among five clones in the southwest side (a) and the southeast side (b).



**Figure 7** Monthly rainfall near the plantation site at Sakaew province from August 1998 to July 1999.

## CONCLUSIONS

In the investigation of seasonal characteristics of wood formation using the knife-cutting method in five superior clones of *E. camaldulensis*, the results were concluded as follows :

1. The radial growth of all 5 clones increased in rainy season and decreased in dry season. In the dry season, the decrease of growth rate was more conspicuous in the latter half.

2. In the comparison among 5 clones, some clones including 1, 2 and 5 showed more conspicuous decrease than 3 and 4 in dry season. Especially, clone 3 showed continuous growth even in the dry season, which might support this clone to be the candidate for the plantation in drier habitat.

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