Seed Moisture Content, Relative Humidity, and Better Storage of Longleaf Pine Seed

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Abstract

The science for preserving the germination of longleaf pine (*Pinus palustris* Mill.) has long been known. In practice, however, the germination of longleaf pine seeds after 1 to 5 years of storage is disappointingly low, resulting in significant financial losses and threatening an already precarious seed supply that is needed for restoration and reforestation. This article discusses the relationship between seed moisture and relative humidity and how that relationship indicates ways to improve the handling of longleaf pine seeds so that germination is maintained in storage. Emphasis is placed on using equilibrium relative humidity testing as a way to improve seed longevity. This paper was presented at the Joint Meeting of the Northeast Forest and Conservation Nursery Association and Southern Forest Nursery Association (Lake Charles, LA, July 18–21, 2016).

Introduction

Trees produce seeds in cycles and, therefore, seeds are commonly preserved during bumper crops for use in years with poor seed crops. For at least 50 years, foresters have understood that the viability of desiccation-tolerant (also called orthodox) tree seeds is preserved by drying the seeds to a moisture content of 6 to 9 percent and then freezing them in sealed, moisture-proof containers (Jones 1966). These moisture and temperature conditions lower the metabolic rate of seeds, thereby putting them in a resting stage where they can remain alive for many years. Of these two storage variables, moisture is the most critical factor (Bonner 2008, Justice and Bass 1978). Barnett (1969) and Barnett and Pesacreta (1993) recommended drying longleaf pine (*Pinus palustris* Mill.) seeds to 10 percent moisture content or lower; they also found the seeds could be stored for up to 7 years at -4 °C (25 °F) and at least 20 years at -18 °C (0 °F). Unfortunately, however, many clients of the USDA Forest Service's National Seed Laboratory (NSL; Dry Branch, GA) have reported unacceptable germination losses after 1 to 5 years of storage, in spite of adhering to these storage recommendations. Thus, some improvement is needed in the operational handling of the seeds to produce a better storage result. To give a good framework for discussing the problem and possible solutions, we will first discuss the concept of seed vigor and moisture relations for longleaf pine seed.

Longleaf Pine Seed Vigor

Germination is the emergence from the seed of a seedling with all essential plant parts. For a particular seed lot, it is expressed as the number of individual seeds out of 100 seeds that germinate. Seed vigor is a concept for determining the relative value of seed lots of comparable germination. It is typically defined using either the speed of germination, the ability to germinate under adverse conditions, or the ability to not lose germination while stored. Understanding vigor as the ability to withstand the loss of germination during storage is the definition most appropriate to the issue with longleaf pine longevity addressed in this article. Therefore, we can think of longleaf seed lots that decrease in germination during storage as not sufficiently vigorous to remain alive. When a seed dies, the switch from living to dead is not immediate. A period of aging and loss of vigor precedes the loss of the ability to germinate. Vigor initially declines at a faster rate than germination does (figure 1). When the vigor loss line in figure 1 is more horizontal, the seeds survive for longer periods of storage. When the vigor line has a steeper slope, the seeds do not survive as well and a drop in germination will be realized sooner.

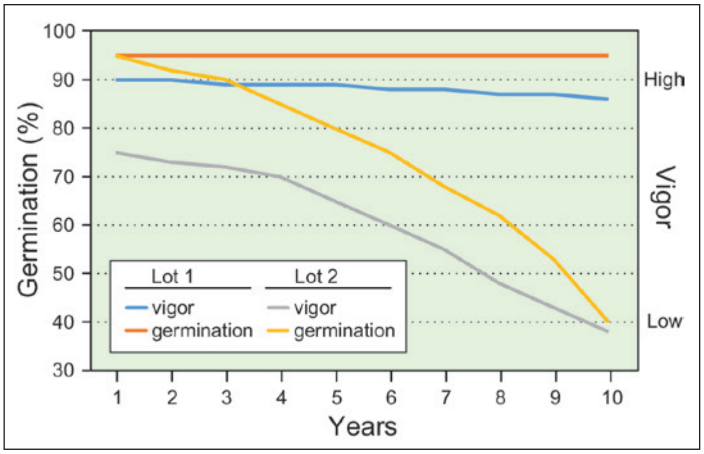

Figure 1. Illustrative graph of seed vigor decrease and germination loss over time. Seed lot 1 is high vigor. Seed lot 2 is lower vigor.

McLemore (1961) reported that longleaf pine seeds must be extracted from the cones within 30 days of harvest for best germination, and that seeds extracted at 60 days postharvest had lower germination. By contrast, high-quality seeds can be extracted from most other conifers, including all other southern pines, for 60 to 90 days postharvest. Therefore, at the very beginning of the process, longleaf pine seeds are demonstrating low seed vigor and a low capacity to remain viable in storage. Interpreting McLemore's findings in light of the vigor graph (figure 1), we can say that at some point between the date of cone collection and 30 days later, vigor begins to decline but not so much that it causes a measureable decline in germination. At some point between 30 and 60 days of cone storage, however, the seeds decline sufficiently in vigor so that germination also declines. The seeds are not dying from a sudden trauma, but from a series of small, cumulative, and increasing losses of vigor over time. Vigor losses initiated during cone storage are likely to increase with time. This process occurs with all pine species, but is more an issue with longleaf pine seeds because they have little or no dormancy. That means they are more physiologically active at harvest time compared with other species of pine seeds. Without internal mechanisms to arrest biological activity, only the control of seed moisture and storage temperature can retard the deterioration process. Another factor influencing the loss of vigor is that longleaf seeds are shed from kiln-dried cones at high moisture levels, which is not the case with other southern pines. To expand on these concepts and improve the preservation of longleaf pine seed quality, three trials were conducted on the relationship between seed moisture content and relative humidity (RH).

Materials and Methods

Two methods were used in these trials to assess seed moisture. The first method was the constant temperature oven method (ISTA 2017) in which seeds are weighed, dried for 16 hours at 103 °C (217 °F), and then reweighed. The weight loss (assumed to be the weight of water originally in the seed) is divided by the original weight of the sample, and the answer is multiplied by 100 to give the moisture percentage on a wet-weight basis. The second method is equilibrium relative humidity (eRH). Testing eRH has been discussed elsewhere (Baldet et al. 2009, Karrfalt 2014) and will be reviewed again here with specific reference to longleaf pine. Figure 2 shows hygrometers (VWR International catalog number 35519-020) attached to test chambers (50 ml Erlenmeyer flasks) containing seeds. Any quality hygrometer can be used, and any vessel can be used to hold the seeds, as long as it can be sealed securely enough to isolate the air inside the vessel from the outside air. Just as seeds adjust their moisture content to changes in RH, air in a closed vessel adequately filled with seeds will adjust to the moisture of the seeds. For example, when seeds are equilibrated at a RH of 30 percent, the air around them in a closed vessel will adjust to RH of 30 percent. The test chamber must be at least one-quarter full; more seed gives more buffer against moist ambient air (50 percent or more RH).

A quality hygrometer costs between $300 and $600. Less expensive hygrometers have not provided reliable service at the NSL. The Rotronic water activity meter (figure 3) (HygroPalm - HP23-AW-A—portable

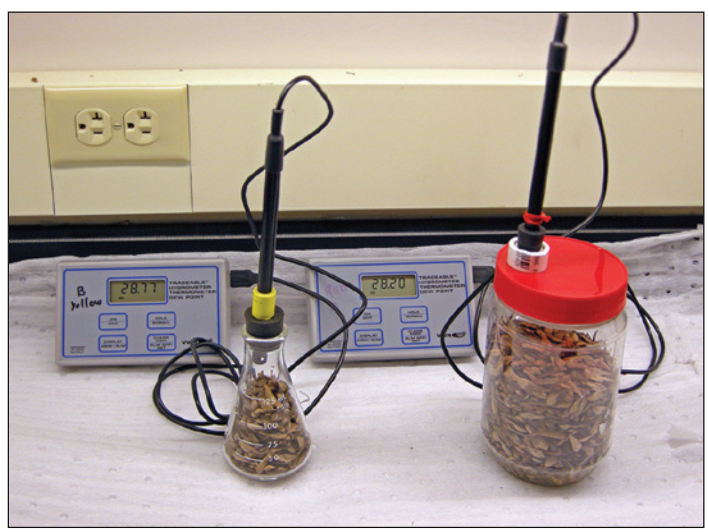

Figure 2. Hygrometers are attached to test chambers to measure seed equilibrium relative humidity. (Photo by R.P. Karrfalt 2017)

Figure 3. Rotronic water activity meter used to measure seed equilibrium relative humidity on small seed samples. (Photo by R.P. Karrfalt 2017)

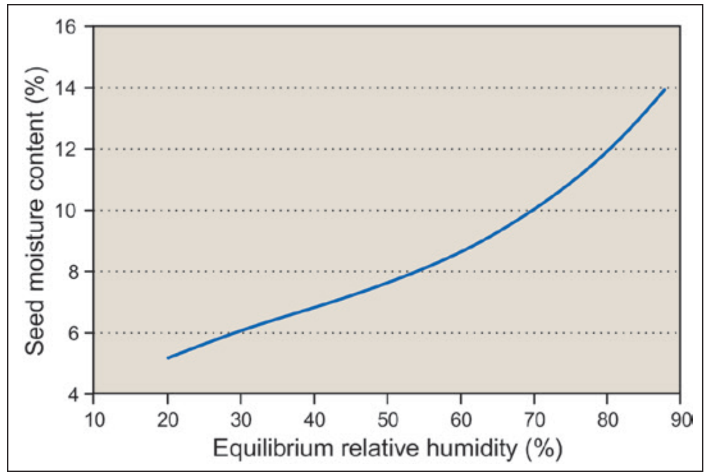

Figure 4. Moisture content of longleaf pine seeds plotted against equilibrium relative humidity.

analyzer, Rotronic USA) is much higher priced ($3,000 or more) than the VWR hygrometer. It was designed for use in the food industry and expresses the RH as water activity which, from a practical point of view in testing seeds, is really only the RH expressed as a decimal rather than as percent. For example, 30-percent RH is equal to 0.30 water activity. The concept of water activity has value in understanding the physiology of the seeds, but for the purpose of determining if seeds are sufficiently dry for storage, it only adds confusion. The Rotronic meter has the advantage of accommodating small samples.

All seed lots used in these trials were samples submitted to the NSL for routine germination tests for nursery sowing and had been in storage for 1 or 2 years.

Trial 1

eRH and seed moisture content are closely related. To describe this relationship, five longleaf pine seed lots were subjected to eight constant RHs, ranging from 20 to 87 percent. Each humidity level was regulated in a closed plastic box by a saturated solution of an appropriate inorganic salt. Seeds were placed in small containers designed for use with the Rotronic water activity meter (figure 3). One open container of each seed lot was then suspended over the salt solution on a fine-screen rack until the weight of the sample had stabilized, and seeds were judged to be at equilibrium with the respective RH. Upon reaching equilibrium, each sample was tested for its eRH with the Rotronic water activity meter and then for moisture content using the constant temperature method. Moisture contents were then plotted against eRH to produce an isotherm (figure 4).

Trial 2

Following a drying period, the initial eRH values were not the same as values taken some time later. To clarify what was happening, a drying trial was done. Seven longleaf pine seed lots, ranging from approximately 33- to 58-percent eRH, were dried on a pressurized dryer (Karrfalt 2014) for 2 hours at 26-percent RH. The seeds were then transferred to test vessels, and the hygrometer was attached and left connected and undisturbed until all readings were complete. The eRH of each seed lot was recorded 10 minutes after drying was stopped and then at 11 additional intervals over the next 120 hours. These eRH values were then plotted against time (figure 5).

Trial 3

To measure how much and how fast seed moisture content might increase with the ambient RH, samples from seven longleaf seed lots were placed into six RH treatments (42 combinations total). The eRH of all lots initially ranged from 30 to 35 percent. The same saturated salt technique described in trial 1 was used to establish the six different RHs. All samples were weighed at the start of the trial and again after 48, 72, and 96 hours to detect moisture increases. From these weights, the moisture content of each sample-humidity combination was calculated.

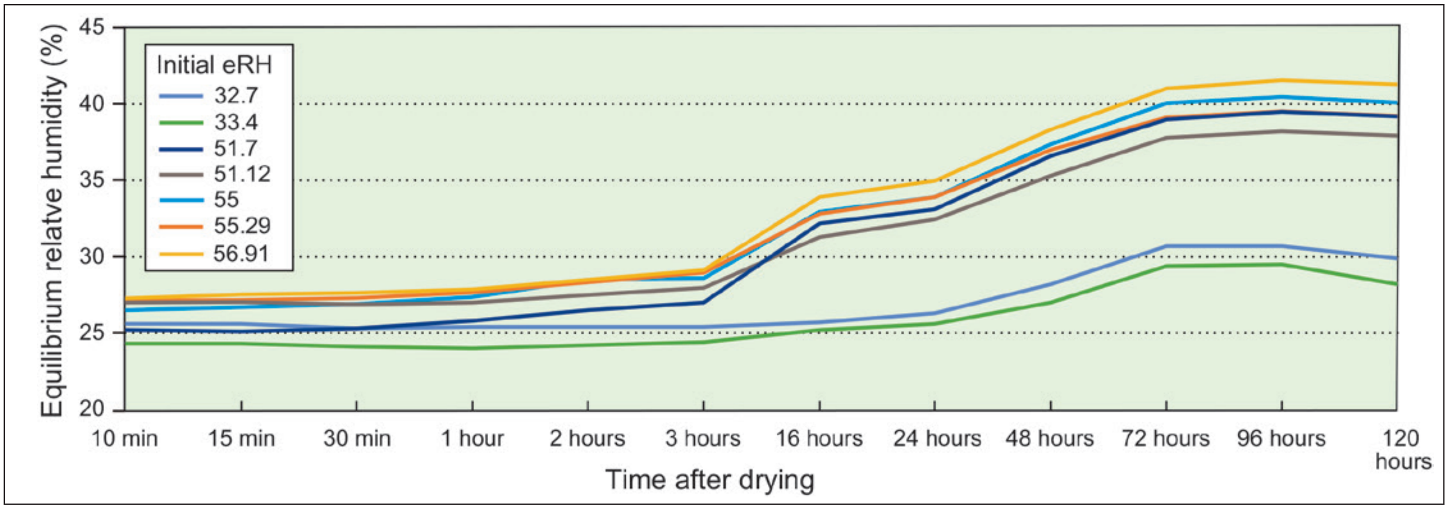

Figure 5. Equilibrium relative humidity readings change until seeds obtain internal moisture equilibrium. Water moves from the inside of the seed to the outer layers until all layers of the seed are in moisture equilibrium.

Results and Discussion

Trial 1

The isotherm shown in figure 4 is typical for seeds of many species. It indicates that 30-percent eRH corresponds to 6-percent moisture content and approximately 60-percent RH corresponds to 9-percent moisture content. In other words, seeds equilibrated at RHs between 30 percent and 60 percent would be in the moisture-content range that Barnett recommended for maintaining longleaf seed germination for 20 years. Equilibrating seeds at RH ≤ 30 percent is not a good idea, because the water removed at those lower RHs is structurally important to the cells, and removal will damage the cell membranes. On the other hand, enzyme activity increases as eRH increases above 30 percent. Therefore, 30 percent should be the best humidity at which to equilibrate longleaf pine seeds for storage because the rate of seed metabolism is as minimal as possible, without damaging the cells by overdrying. This optimal humidity was also apparently the case with sagebrush (*Artemesia tridentate* var *wyomingensis* Beetle & Young) (Karrfalt and Shaw 2013), another desiccation-tolerant species that is frequently short-lived in storage. At storage temperature of -8 °C (17.6 °F), sagebrush seeds at 40-percent eRH did not survive as well as seed equilibrated at 30-percent eRH. Because 30-percent eRH appears to be an optimal seed moisture level for storage, it is logical to use eRH as a moisture test. As will be explained later, moisture content can be less precise for indicating that the optimal moisture status has been achieved.

Trial 2

After drying longleaf pine seed for 2 hours with air at 26-percent RH, all seed lots in trial 2 had the same eRH, of 26 percent, for at least 1 hour. Those seed lots, which were initially the driest (predry eRH of 32.7 and 33.4 percent), continued to test at 26 percent for 3 hours and changed very little, even at 24 hours. All seed lots took until 72 hours to present their true eRH, one that did not change during the next 24 hours. These results imply two things. The first implication is that recently dried seeds may give a false reading lower than the true eRH, requiring subsequent readings to be sure the values are correct. If the readings are not verified, seeds might be put into storage at moistures higher than optimal to preserve germination and vigor. The second implication is it can take up to 3 days for water deep inside the seed to work its way to the surface of the seed coat and evaporate (figure 6). In other words, it can take 3 days, perhaps longer, to dry longleaf seeds to a moisture content that is safe for storage. This finding is especially important on the initial dry of seeds just freshly extracted from the cone, as they are known to be 10-percent moisture content or higher, or in terms of eRH, above 70 (figure 4). Because it is the first time seeds are being dried, they will likely have moisture in the layers of gametophyte furthest from the seed coat and the embryo. Meters that estimate moisture content will be discussed in the following but are mentioned here to say that they, too, can produce false readings because the surface layers of the seed can be drier than the interior portions.

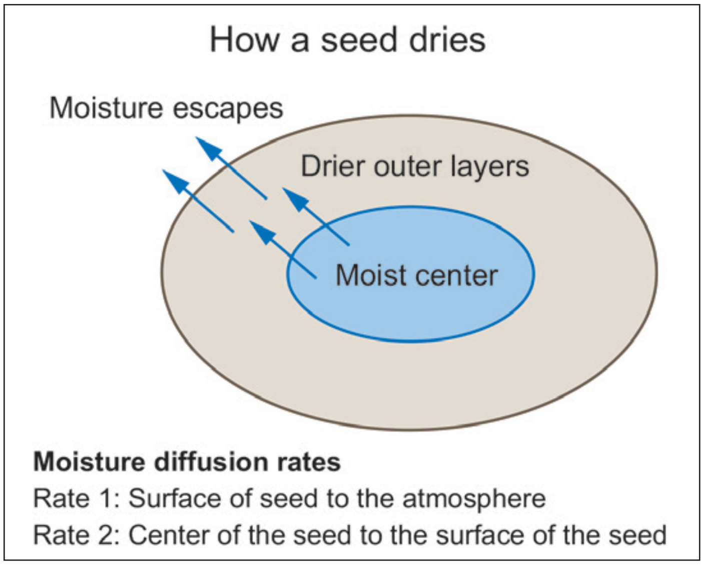

Figure 6. Moisture moves within the seed from areas of higher moisture to areas of lower moisture until the seed is internally at moisture equilibrium.

Trial 3

From trial 3, we see how much and how fast seed moisture content increases under different ambient RHs (table 1). When ambient conditions are near 30 percent, the seeds remain near their ideal moisture content to remain vigorous and able to germinate. As RH increases, seed moisture content also increases. When ambient conditions are damp and RH exceeds 70 percent, seeds can become borderline or even reach dangerous moisture contents in as little as 48 hours. Therefore, it is very important not to leave seeds exposed to humid air unless necessary to work with the seeds for short periods of time. Ideally, seed moisture should be monitored frequently each day the seeds are exposed to ambient conditions. Drying should be done as soon as possible when eRH values are 40 percent or above to bring the seeds back to the optimal eRH of 30 percent. Never make the assumption that once the seeds are dry they will remain dry. Seeds are continually adjusting their moisture level to the humidity around them.

Table 1. Mean moisture content of seven longleaf pine seed lots over time when exposed to air of six different relative humidity levels.

	Mean moisture contents		
RH (%)	48 hours	72 hours	96 hours
33	6.4	6.4	6.5
43	7.1	7.2	7.3
53	8.0	8.5	8.6
76	9.0	9.6	10.2
84	9.7	10.3	11.3
100	13.8	15.3	18.2

RH = relative humidity.

Moisture Meters and Longleaf Pine Seed

Moisture meters (figure 7) have been used to test longleaf pine seed moisture content. To use a moisture meter, the seeds are poured into a chamber between two electrodes. Then the electrodes are energized and either the conductance or resistance between them is measured. The meter reading is converted to moisture content using a previously constructed conversion chart. Accurate readings from these meters require that the seeds make solid contact with the electrodes. Therefore, only well-cleaned seeds can be tested in moisture meters. Monitoring moisture in unfinished raw seeds with a moisture meter is not possible without first cleaning the test sample to the same degree as the finished product. In addition, raw seeds will still contain empty seeds that will bias the moisture meter reading toward moisture contents lower than is true for the full seeds. Although moisture meters are available, a hygrometer testing eRH is more reliable because it is able to accurately test the seeds without bias, regardless of the state of cleaning, thereby enabling frequent, if not nearly continuous, seed-moisture measurements.

Figure 7. Seed moisture meter that has been used to estimate seed moisture content. (Photo by R.P. Karrfalt 2016)

Quality Control for eRH Testing

In addition to regular checks of seed eRH, checks on the hygrometer need to be made on a regular schedule. During the seed-cleaning season, the hygrometer should be checked daily and before use at other times of the year. The check is made by filling a test vessel about halfway with a saturated solution of magnesium chloride and then measuring eRH. Magnesium chloride is used because it will create an RH very close to 33 percent between the temperatures of 20 and 30 °C (68 and 86 °F). Choosing the 33-percent test value insures the meter is reading correctly for measuring the target value of 30-percent eRH with the seeds. Care needs to be taken not to get the solution on the hygrometer probe, as salt on the probe could create a bias when measuring seed eRH or damage the sensors. The hygrometer should read between 31 and 35 percent at the check; if the hygrometer fails it should be replaced or recalibrated.

Conclusion

eRH testing offers a new opportunity to increase the storage life of longleaf pine seeds. First, the understanding of seed moisture relations provided by eRH suggests that operationally produced seed lots might maintain better germination if moisture is reduced to the driest moisture condition recommended by Barnett. Theoretically, 30 percent eRH (6 percent moisture content) should be superior to 60 percent eRH (9 percent moisture content). Second, eRH testing can provide accurate moisture evaluations on raw seeds, which facilitates early detection of high seed moisture through frequent evaluations throughout the cleaning process, and subsequently the immediate drying of any seeds that are not at optimal moisture levels.

Key points for using eRH to improve longleaf pine seed storability are as follows:

1. Longleaf pine seeds are reduced in storability the longer they are held in the cone.
2. Seeds just extracted from the cone will be at high moisture content and will require immediate drying.
3. Germination is anticipated to be best-preserved by drying seeds to 30-percent eRH and maintaining seeds at that level.
4. Above 30-percent eRH, seeds are slowly and silently dying (losing vigor), which later shows up as an unexpected loss of germination, even in seeds between 6- and 9-percent moisture content.
5. eRH can be accurately measured on raw, uncleaned seeds as well as finished seeds.
6. Hygrometer accuracy should be checked every day of use with a saturated solution of magnesium chloride.
7. Freshly dried seeds might test as being drier than they actually are. You should repeat measurements in 16 to 24 hours to verify the accuracy of eRH test.
8. You shoud never assume dry seeds are staying dry. Test the eRH frequently and dry as needed.
9. Figure 8 provides a flow chart to guide the management of moisture in longleaf pine seeds.

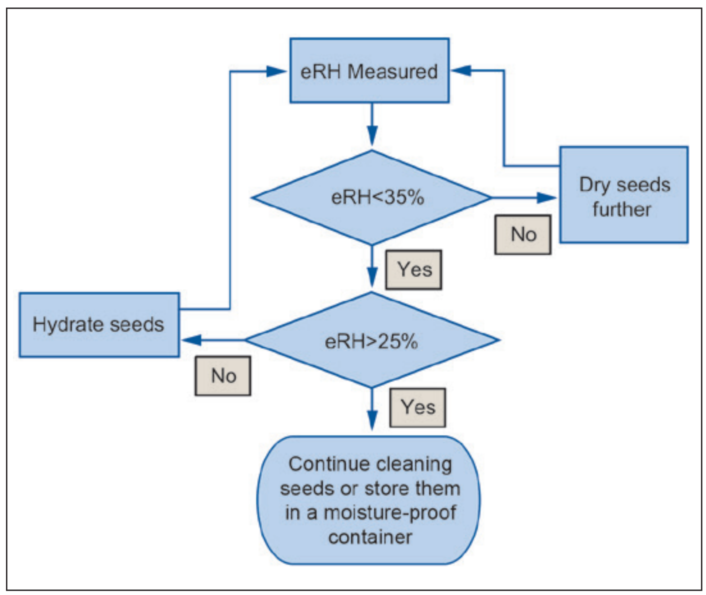

Figure 8. Flow chart of actions and decisions needed to maintain longleaf pine seeds at optimal moisture status to preserve germination.

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