

## THE MICROELEMENT MOLYBDENUM (Mo) IN NUTRITIONAL SUPPLEMENT FOR BEES

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### Abstract

The aim of this work was comparative testing in bees' food of nutritional supplements enriched with biologically active substances and elaboration of efficient proceedings feeding bees during harvesting deficient in nature. As biologically active substances have been used, some coordination organic compounds containing rare trace elements in particular Mo. Testing was conducted in spring (April 1 to 21) in three batches bee families. Family from all the batches received energizing nutrient mixture every 2 days for 3 weeks. For families from batch II the nutrient supplement was enriched with bioactive patented "Apispir + Cr" obtained from cyanobacteria *Spirulina platensis* biomass, cultivated in the presence of coordinative organic compound Sulfate chromate of dodecahydrate potassium  $[KCr(SO_4)_2 \cdot 12H_2O]$ , and for batch III - with bioactive supplement of the coordinative organic compound  $(PPh_4)_2 [Mo_2O_4(EDTA)] \cdot 2.5H_2O$  - [tetraoxoetilendiaminotetraacetat molybdenum (V)] bis-(tetraphenylphosphonium) di-hemihydrate named as "AA20Mo". Research has shown that biologically active substances of mentioned above coordination compounds, contributes significantly to the activation of family reproductive functions (queen prolificacy, amount of capped brood, family strength), fortification of the insects organism immunity (increased disease resistance and brood viability) and increase the overall productivity of bee colonies. Beneficial influence of coordinative organic compounds on vital functions of bees is explained not only by the action of rare trace elements, but also complex action of all biologically active substances of their complicated molecular structure with very close ties to their structural and stable, including ions complexes of ligands and metal ions valence radicals modified and enhanced properties of cell membranes penetrating living tissues of the organisms of the bee. It was found that feeding of bee families on deficiency harvesting period in nature nutritional supplements enriched with coordination organic compounds containing rare micronutrients raises the level of development of morph-productive characters of bee families. Using in bees feeding the energizing nutritional supplement enriched with organic compound containing the coordinating AA20Mo (with Mo<sup>v</sup>) helps to increase: queen prolificacy and amount of capped brood with 10.5%, family strength with 11.9%, brood viability and disease resistance with 2.2-4.3%, the amount of accumulated bee bread in the nest with 21.8%, amount of increased wax combs with 39.3% and honey production accumulated in the nest with 20.0%.

**Key words:** feeding, the bees, nutritional supplements, rare microelements, Mo, Cr

### INTRODUCTION

The problem of feeding of bees during deficient harvesting in nature is a permanent concern of beekeepers and experts in the field [2, 3, 8, 9, 14, 15, 18]. After the winter, early spring bee colonies are usually convalescent as a result of confrontation with the negative impact of different weather. The bees' body there is a bioactive nutrient deficiency,

particularly of rare trace elements which have a catalytic role in the physiological processes of vital activity of bees, performing multiple functions in bee organism at the cellular level, entering in the composition of enzymes and hormones playing a decisive role in metabolism [3]. Insufficiency of biologically active substances, especially in trace elements, leads to the retention of reproductive functions activity, weakening resistance to diseases of bee families and decrease their productivity [12].

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The main natural sources to supply the bees' organism with nectar and pollen are micronutrients, collected from plants flowers by bees. According to scientific information [1, 9], honey and pollen are more than 30 micro-macro elements, including trace elements, which have an enormous role in physiological metabolism of living organisms. Among them, molybdenum (Mo), is a rare trace mineral, is found in very small quantities, up to 14 mg% (after Kerkvliet, 1989, quoted by Cristina Mateescu [9]), but its role in the metabolism of trace substances in the organism of bees until now, it is not known.

Due to the fact that early spring (poor harvesting period in nature), most beekeepers feed bees families with sugar syrup, the composition of which the majority necessary trace elements are missing, identifying available sources of trace elements for enriching the ration supplements bees, it becomes a serious problem.

There are known a number of proceedings for the feeding of bee families with sources of trace elements [16, 20, 21], in a mixture with sugar syrup, pollen substituents, minerals, amino acids and vitamins set, once distributed into the hive 12 days, for 36 days. The disadvantages of these proceedings are that they are expensive, too complicated nutritional composition of mixtures, processes for preparing and implementing them is too long and trace elements added as a mineral salts are poorly digestible by bees, easily oxidized, causing disturbances digestive tract of bees.

For these reasons, the viewfinder of researchers of sources of biologically active substances, in particular, rare trace elements, was reoriented towards remedies insufficiently understood fundamentally new properties, such as coordination organic compounds. According to Заозерский И.Н., 1965 [17], a number of rare-earth elements, which are found in plant and animal tissues, enter into the coordinative complex compounds. The coordination compounds of metals are bonded in the complex have a valence change and are part of the enzymes, in particular the oxidative.

Our previous research [4-7, 12, 13] have demonstrated that feeding of bees with nutritional supplements enriched with trace

elements introduced in the form of solutions of coordinative organic compounds with changed valence, the cultivation of *Spirulina platensis* biomass, have a stimulatory impact to the development of morph-productive characters of honeybee families. The essence of the technique of these proceedings is that the feeding of bee families in spring with a mixed solution of 1% mass strain biomass extract from *Spirulina platensis* CNM-CB-02 and 50% sugar syrup in the ratio of 1: 500, respectively, *Spirulina platensis* strain was cultivated in the presence of the coordinative organic compounds, and the feeding of bees with the mixture is performed in an amount of 100 ... 130 ml bees frame, every 2 days for two weeks.

The disadvantage of these proceedings is that that the technology of obtaining the strain *Spirulina platensis* extract of biomass cultivated in the presence of coordinative organic compounds is too complicated and expensive, and the use efficiency of trace elements from coordinative organic compounds administered, is relatively low.

In this context, identification of new sources of micronutrients, especially rare ones like Mo, has a particular interest. The aim of this work was comparative testing in bees food of nutritional supplements enriched with biologically active substances of coordinative organic compounds containing rare trace elements, in particular Mo, in and elaboration of efficient processes feeding bees during harvesting deficient in nature.

## MATERIAL AND METHODS

The research was performed on families of bees *Apis mellifera Carpatica*, at the experimental apiary of Institute of Zoology of the Academy of Sciences. Apiary is located on stationary in a clearing of the forest, close to its edge. The main melliferous sources in this area are white acacia, lime-tree and spontaneous flora, including yellow melilot.

In special experiences in bees feed was tested in spring (April 01 to 21), during the poor harvest in nature, a nutrient mixture of 50% sugar syrup enriched with bioactive supplement coordinative organic compound:  $(\text{PPh}_4)_2[\text{Mo}_2\text{O}_4(\text{EDTA})] \cdot 2,5\text{H}_2\text{O}$  - [tetraoxo-etilendiaminotetraacetat molybdenum(V)]

*bis-(tetraphenylphosphonium)di-hemihydrate* an aqueous solution with a concentration of 1 mg% (named as "AA20Mo"), which was mixed with sugar syrup in a ratio of 2:100, was administered directly into the feed of bees and bee families feeding the nutrient was carried out at 100 ... 130 ml of each frame interval inhabited bees, every 2 days for three weeks.

To estimate the efficiency of the process of feeding bees with up-nominated nutritional supplement, were carried out comparative testing its experiences on bee colonies formed into three batches, each batch by 16 families, including: batch I - control, in which the bees were fed with sugar syrup only 50%, batch II - prototype, the bees received in feed sugar syrup enriched with patented nutritional supplement "Apispir + Cr" (MD 476 Z 2012.09.30) obtained from biomass of *Spirulina platensis* cultivated in the presence of coordinative organic compound *Sulfate chromate of dodecahydrate potassium* [KCR (SO<sub>4</sub>)<sub>2</sub> · 12H<sub>2</sub>O], and batch III - actual experimental, the bees received in feed sugar syrup supplement enriched "AA20Mo".

After the spring stimulation, at 45 days of the start of the experiment and the first harvest (70 days after the start of the experiment), the bees families were evaluated after morph-productive following characters: the queen prolificacy, amount of capped brood, family strength, resistance to disease, brood viability, amount of increased wax combs in the nest, the amount of accumulated bee bread in the nest.

Determining the level of characters development of bee families morph-productive was carried out as required by Zootechnical norm for evaluation of bee families, growth and certification of apiarian genitor material, approved by Government Decision no. 306 of 28.04.2011 (OJ no. 78-81 of 13.05.2011, art. 366) [10].

The amount of capped brood in nest was determined with Netz frame by measuring the number of squares (5 x 5 cm) occupied by capped brood, which have been multiplied by 100, resulting in total capacity brood cells.

Prolificacy queen (eggs / 24 hours) was determined at the first harvest, by dividing the

number of cell with capped brood to 12 (duration of development cycle, days), resulting in the number of eggs laid in 24 hours.

Strength of bee family was determined by multiplying the number of intervals between frames, uniformly occupied with bees, the coefficient 0.25 for standard frame Dadant (435x300 mm), resulting in the amount of existing bee nest when assessing (the first harvest).

Resistance to disease was identified by observing hygienic behavior of bees' families. For this, a portion of the honeycomb from each family nest, on a square of 5 x 5 cm (100 cells) in the corners marked with matches, was killed brood at stage capped (pupa) by pricking with a fine needle through the cells cap. After 24 hours, the comb was introduced in the nest, and the number of brood removed from the cells was appreciated. The ratio of removed brood and initially killed, on the marked surface of the comb, expressed as a percentage, presented resistance to disease.

The viability of the brood was evaluated by marking each bee family with matches in the corners of a portion of honeycomb compact egg laying 400 cells (10 x 10 cm). After 4-5 days, the larvae were counted on the surface of cells marked. The ratio of brood cells and total cells on the marked surface presented the viability of the brood, expressed as a percentage.

The amount of the bee bread in the nest was determined by measuring with frame Netz the of the number of square (5 x 5 cm) occupied with bee bread cells that have been multiplied by 100, resulting in the total number of cells with bee bread.

The amount of increased wax in nest was determined by multiplying the number of newly grown combs with wax standard weight of a comb (120 g).

The amount of honey accumulated in the nest was determined by the method of Szabo T.I 1989 [11], by weighing each hive before harvesting and the end of harvest, the difference being the amount of honey gathered in the nest during this period.

The data obtained as a result of the research were processed statistically using computer software "STATISTICS-6" and

appreciated their certainty, according to variation biometric statistics, after the methods of Плохинский Н.А. 1969 [19].

## RESULTS AND DISCUSSIONS

Results of testing of supplements in bee feed, enriched with aqueous solutions

containing coordinative organic compounds with rare trace elements, have demonstrated that these (supplements) had generally a beneficial action on the vital activity of bee families and growth, in particular, their productivity (Table 1).

Table 1 The results of testing in bees feed (the first harvest) of nutritional supplements enriched with coordinative organic compounds, containing rare microelements

The experimental batch and bioactive element	The number of families in batch, N	Average value of the character at 70 days from the start of the experiment, $M \pm m$	The difference from control batch,		Certainty coefficient of the difference, $t_d$
			d	%	
The queen prolificacy, eggs/24 h					
Batch I (control)	16	1590 ± 20	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	1673 ± 14	+ 83	5.2	3.4**
Batch III ( <i>AA20Mo</i> )	16	1757 ± 15	+167	10.5	6.7***
The number of capped brood, hundreds cell					
Batch I (control)	16	190.8 ± 2.4	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	200.7 ± 1.6	+ 9.9	5.2	3.4**
Batch III ( <i>AA20Mo</i> )	16	210.8 ± 1.7	+20.0	10.5	6.8***
Family strength, kg					
Batch I (control)	16	3.20 ± 0.02	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	3.28 ± 0.04	+ 0.08	2.5	1.7
Batch III ( <i>AA20Mo</i> )	16	3.58 ± 0.05	+ 0.38	11.9	7.1***
Resistance to disease, %					
Batch I (control)	16	88.4 ± 0.4	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	90.5 ± 0.3	+ 2.1	2.4	4.2***
Batch III ( <i>AA20Mo</i> )	16	92.2 ± 0.4	+ 3.8	4.3	6.7***
Brood viability, %					
Batch I (control)	16	89.3 ± 0.3	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	90.4 ± 0.3	+1.1	1.2	2.6**
Batch III ( <i>AA20Mo</i> )	16	91.3 ± 0.2	+2.0	2.2	5.5***
The amount of the bee bread, hundreds cell					
Batch I (control)	16	90.5 ± 1.8	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	104.1 ± 1.5	+13.6	15.0	5.8***
Batch III ( <i>AA20Mo</i> )	16	110.2 ± 2.7	+19.7	21.8	6.1***
The amount of the wax, kg					
Batch I (control)	16	0.28 ± 0.01	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	0.34 ± 0.01	+0.06	21.4	4.3***
Batch III ( <i>AA20Mo</i> )	16	0.39 ± 0.01	+0.11	39.3	7.8***
The amount of honey, kg					
Batch I (control)	16	11.62 ± 0.40	-	-	-
Batch II ( <i>Apispir</i> + <i>Cr</i> )	16	13.24 ± 0.40	+1.62	13.9	2.9**
Batch III ( <i>AA20Mo</i> )	16	13.94 ± 0.36	+2.32	20.0	4.3***

Remark: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

Be mentioned that in the 45 days of the start of the experiment, batch of bee families which received the food supplements enriched with coordinative organic compounds containing rare micronutrients both *Apispir* + *Cr* and with *AA20Mo* had only a rising trend compared with the control batch, the level of character and morph-productive value

contained within the 1.7 to 4.0% ( $P > 0.1$ ). Since that the differences certainty factor recorded was below the threshold of probability theory forecasts zero without error after student [17], meaning beneficial effect at this stage (45 days after the start of the experiment), wearing a only character trend.

However, after 70 days from the beginning of the experiment (see table at the first harvest), the beneficial influence of biologically active organic compounds coordination both *Apispir* + *Cr* supplement and the supplement *AA20Mo* became significant and the coefficient of certainty of difference between the value of morph-productive characters of bee families in the experimental and control batches reached the highest threshold, according to probability theory of forecasts without error.

The queen prolificacy from bee families was identical in all batches at the beginning of experience, thereafter, at first harvest, significantly increased compared with control batch, the experimental batches whose families have received food supplements enriched with both *Apispir*+*Cr*, and with the coordinative organic compound *AA20Mo*. The most pronounced growth of queen prolificacy was registered in the batch III, where bees were fed in spring (April), with energy nutritional supplement enriched with micronutrients coordinative organic compound containing rare microelement *AA20Mo*. Queen of bees families in this batch certainly exceeded after prolificacy, their congeners from the control group - with 167 eggs or 10.5% ( $t_d = 6.7$ ;  $P < 0.001$ ). Be mentioned that the difference compared with controls, the prolificacy of queen in bee families batch III, who received food nutritional supplement enriched with the coordinative organic compound containing rare trace elements (*Mo*) was 2.0 times higher than the difference queen bee families in batch II, who received the food supplement enriched with *Apispir* + *Cr* ( $P < 0.001$ ).

**The amount of brood capacity** as determined by the prolific queen, was also positively influenced by nutritional supplements enriched with both biomass extract *Apispir*+*Cr*, and with the coordinating organic compound containing rare trace elements such as *AA20Mo*.

Thus, the experimental bee families vastly exceeded by this character at first harvest the families from control batch.

The biggest increase in the number of capped brood, compared to control batch, was registered in bee families of batch III, who received food nutritional supplement enriched with the coordinative organic compound containing rare trace elements *AA20Mo*. The difference in capacity of increase the number of capped brood in bee families of this batch, compared to the control batch, was 20.0 hundred cells, or 10.5%. This difference is significant, with the highest threshold of probability of certainty forecasts, without error by Student ( $t_d = 6.8$ ;  $P < 0.001$ ).

More obvious this increase of development level of *morph-productive* characters of bee families from experimental batches can be viewed in histogram (figure 1).

From the diagram is seen that the level of all the morph-productive characters of bee families from experimental batches II and III is significantly higher than the control group.

**The strength of bee families** was identical in all groups at the beginning of experience, thereafter, at first harvesting, significantly increased, compared with control batch, the experimental batches, whose families have received in food nutritive supplements enriched with both biomass extract *Apispir*+*Cr*, and with the coordinative organic compound containing rare trace elements (*Mo*). The most pronounced increase in strength of bee families was registered in batch III, the bees were fed in spring (April) with energetic supplement enriched with coordinative compound *AA20Mo*. Bee families from this batch certainly exceeded after strength, their congeners from the control batch - 0.38 kg or 11.9% ( $t_d = 7.1$ ;  $P < 0.001$ ). Be mentioned that the difference, compared with controls, bee families strength in batch III, who received in food nutritional supplement enriched with the coordinative organic compound containing rare trace elements (*Mo*) was about 4.7 times more, than to the families of bees in batch II who received the food supplement enriched with *Apispir* + *Cr*.

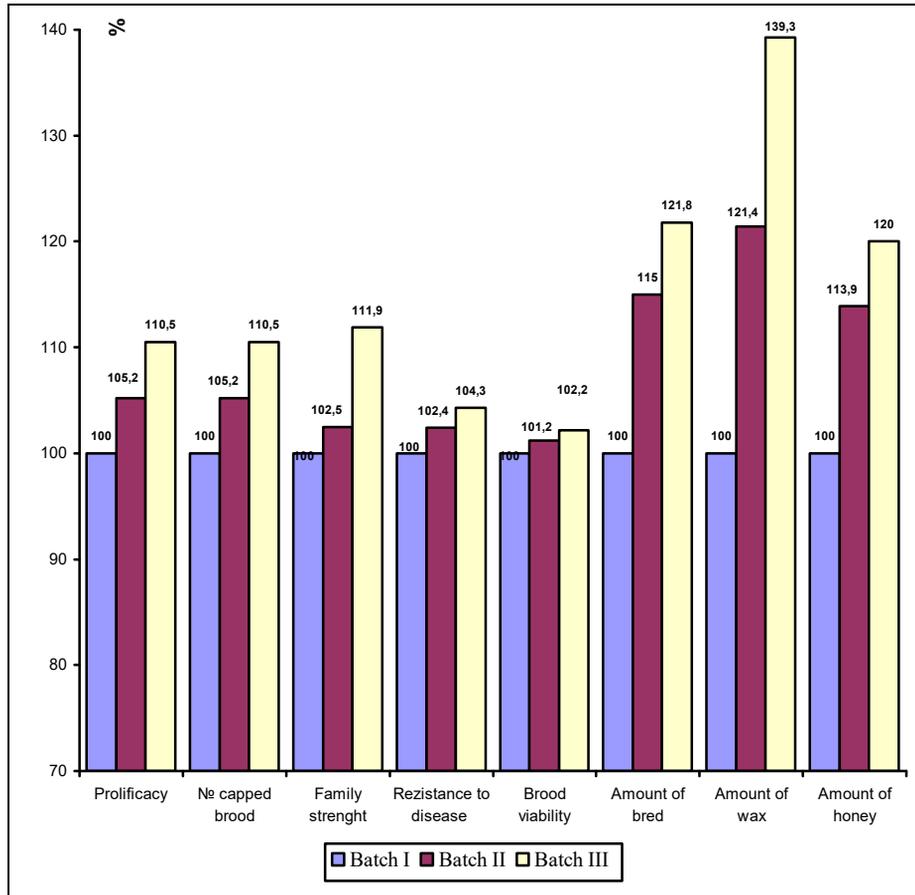


Fig. 1 Level of morph-productive characters from bee families, compared to control group

**Resistance to diseases** of bees families, is a biological features determined hereditary, however, it was influenced by external factors, among which the most important is nutrition. The experiment results showed that at the first harvest experimental bee families have greater resistance to disease compared with controls. The highest resistance to disease was registered bee families the batch III, who received food nutritional supplement enriched with the coordinative organic compound containing rare trace *A420Mo*. After the value of this character, bee families from experimental batch III exceeded their congener from control batch by 3.8 percentage units or 4.3% ( $t_a = 6.7$ ;  $P < 0.001$ ). Given that that biological variability of this biological character is very narrow, the significance of this difference (small at first sight, absolute size) is quite high and corresponds to a high threshold of

certainty, according to theory probability of forecasts without error.

**The amount of bee bread** accumulated in the nest was also positively influenced by the nutritional supplements enriched with the extract of biomass as *Apispir+Cr*, and with the coordinative organic compound containing rare trace elements such as *Mo*. Thus, the experimental bee families vastly exceeded by the level of this character in the first harvest their congeners from the control batch. The biggest increase in the amount of accumulated bee bread in the nest, compared to the control batch, was registered at bee families the batch III, who received food nutritional supplement enriched with the coordinative organic compound containing rare trace *Compound+Mo*. The difference in the quantity of bee bread growth in bee families in this batch, compared to the

control batch, is 19.7 hundred cells or 21.8%. This difference is significant with the highest threshold of certainty probability forecasts without error ( $t_d = 6.1$ ;  $P < 0.001$ ).

**The amount of wax** increased on combs in the nest, compared to the other *morph-productive* characters, was most positively influenced by nutritional supplements enriched with both biomass extract *Apispir+Cr*, and with the coordinative organic compound containing rare trace elements such as *AA20Mo*. Thus, the experimental bee families vastly exceeded by the level of this character in the first harvest their congeners from the control batch. The biggest increase in the amount of wax in the nest, compared to the control, was registered bee families the batch III, who received food nutritional supplement enriched with the coordinating organic compound containing rare trace elements *AA20Mo*. The difference in growth compared, to the control batch, the amount of accumulated wax in nest at bee families in this batch was 0.11 kg, or 39.3%. This difference is significant with the highest threshold of certainty probability forecasts without error ( $t_d = 7.8$ ;  $P < 0.001$ ).

**The amount of honey** accumulated in the nest, being the most important morph-productive character, was also positively influenced by nutritional supplements enriched with both biomass extract *Apispir+Cr*, and with the coordinative organic compound containing rare trace elements such as *AA20Mo*. Thus, the experimental bee families II and III significantly exceeded after the level of this character of production, at the first harvest, their congeners from control batch, respectively, 1.62 and 2.32 kg or 13.9 to 20.0% ( $P < 0.01$  and  $P < 0.001$ ). Of these two batches, the highest increase in the amount of honey accumulated in the nest, compared to the control, was registered at bee families from the batch III, who received in food nutritional supplement enriched with the coordinative organic compound containing rare microelements *AA20Mo*. The difference in increase of the amount of honey accumulated in the nest of bees' families in this sample is higher compared with group II, of 1.43 times.

Therefore, generalizing results of research of development level of *morph-productive* characters of bee families in the testing experiment of the coordinative

organic compounds in bees food, during deficient harvesting period in nature, can conclude that biologically active substances from the mentioned above compounds, certainly contributes to the activation of family reproductive functions (queen prolificacy, amount of capped brood, family strength), fortifying immunity of insect organism (increased disease resistance and viability of brood) and a substantial increase in overall productivity of bee families.

Beneficial influence of coordinative organic compounds on vital functions of bee families is explained by us, not only by the action of rare trace elements, but also by complex action of all biologically active substances with very complicated molecular structure, with their structural links to close and stable, including ion complexes of ligands and metal ions radicals with modified valence and enhanced properties of cell membranes penetrating living tissues of the organism of the bee.

## CONCLUSIONS

1. The feeding of bee families with nutritional supplements enriched with organic compounds coordination with rare trace element content, in deficient harvesting period in nature (early spring), contributes to the increase of development level of morph-productive characters of bee families of *Apis mellifera*.

2. Using in the feeding of bees of energizing nutritional supplement enriched with coordinative organic compound *AA20Mo*, containing rare microelement (*Mo*), contributes to the increase, compared to the control batch, of the queen prolificacy bees and amount of capped brood by 10.5%, strength of bee families with 11.9%, brood viability and disease resistance of the colony with 2.2 to 4.3%, the amount of accumulated bee breeding nest by 21.8%, the amount of wax increased on frames by 39.3% and honey production accumulated in the nest by 20.0%.

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