Feeding preference and performance of *Helicoverpa zea* (Lepidoptera: Noctuidae) larvae on various soybean tissue types

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Abstract

*Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) feeding preference and performance on soybean tissue types is poorly understood. We assessed preference by looking at feeding behavior and resulting performance of 2nd and 4th instar *H. zea* larvae on leaves, flowers, and pods in no-choice and choice assays. Consumption indices were calculated and observed feeding behaviors were used to indicate preference; survival, growth rate, and larval body mass were recorded as measures of performance. Second instars performed better when fed leaf tissue than when fed other tissue types. In no-choice assays, 32% of 2nd instars that fed exclusively on newly emerging trifoliates reached the pupal stage, and 50% of those that fed exclusively on fully emerged leaf trifoliates survived to pupation. Early instar survival was poor (ranging from 0 to 3%) on all other tissue types, including flowers, stems, and pods. However, when given a choice of tissue types throughout their larval lifetime, 2nd instars preferred to feed on newly emerging trifoliates and early developing pods, consuming on average 51 and 38%, respectively, of each tissue type. In no-choice assays, 4th instars performed best on pods with fully developed seeds; however, when presented with a choice throughout their lifetime, late instars did not feed at a higher rate on any single tissue type. If *H. zea* exhibits similar behavior under field conditions, information on preference can be used to inform management practices and may aid in the development of conventionally bred and transgenic varieties.

Key Words: observed feeding; larval survival; corn earworm

Resumen

La preferencia de alimentación y su comportamiento sobre los tipos de tejidos de soja por *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) es poco conocido. Evaluamos la preferencia al observar el comportamiento alimenticio y el desempeño resultante de larvas de *H. zea* del segundo y cuarto estadios en hojas, flores y vainas durante pruebas de no-elección y de elección. Se calcularon índices de consumo y se observaron el comportamiento de alimentación para indicar su preferencia; se registraron la sobrevivencia, tasa de crecimiento y masa corporal larval como medidas de rendimiento. Los segundos estadios indicaron mejor cuando se alimentaron con tejido foliar que cuando se alimentaron con otros tipos de tejidos. En los ensayos de no-elección, el 32% de los 2º instares que se alimentaban exclusivamente de hojas trifoliadas recién emergentes sobrevivieron hasta el estadio de pupa, y el 50% de los que se alimentaron exclusivamente de hojas trifoliadas totalmente emergidas sobrevivieron hasta el estadio de pupa. La sobrevivencia de los primeros instares fue baja (del 0 al 3%) en todas las otras clases de tejidos, incluyendo flores, tallos y vainas. Sin embargo, cuando se les dio la elección de los tipos de tejidos durante su vida de larvar, el 2º instar prefirió alimentarse sobre las hojas trifoliadas recién emergidas y las vainas que se desarrollaron de forma temprana, consumiendo un promedio del 51% y 38%, respectivamente, de cada clase de tejido. En los ensayos de no-elección, las larvas del 4 estadio se desempeñaron mejor en las vainas con semillas completamente desarrolladas; sin embargo, cuando se les presentó una opción a lo largo de su vida, los últimos instares no se alimentaron a una tasa más alta en ninguna sola clase de tejido. Si *H. zea* exhibe un comportamiento similar bajo condiciones de campo, la información sobre preferencia puede usarse para informar las prácticas de manejo y puede ayudar en el desarrollo de variedades convencionales y transgénicas.

Palabras Clave: alimentación observada; sobrevivencia de larva; gusano del maíz; gusano elotero

*Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), commonly referred to as the bollworm, corn earworm, and tomato fruitworm, has been recorded as a pest on soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), since the early 1900s (Hardwick 1965) and can cause widespread damage to this crop, especially late in the growing season. *Helicoverpa zea* may feed on leaves, pods, and seeds and is often difficult to control if it feeds internally on reproductive structures (Nuenzig 1963; Hardwick 1965; Sharma 2005). In the southern USA, corn (*Cyperales: Poaceae*) is a preferred *H. zea* host (Hardwick 1965; Martin et al. 1976; Sharma 2005) although adults disperse to other crops, including soybean, after corn begins to mature (Hardwick 1965; Terry et al. 1987a,b; Head et al. 2010). Infestations by *H. zea* can reduce soybean yields (Eckel et al. 1992a), but the extent of this impact varies with infestation timing and density. In the upper southeastern USA, the most serious infestations of *H. zea* in soybean happen in late Jul and early Aug following their emigration from corn.

Soybean development can be divided into vegetative (pre-bloom) and reproductive growth stages. Reproductive growth stag-
es can be further divided into flowering (R1–R2), pod development (R3–R4), seed development (R5–R6), and seed maturation (R7–R8) growth stages (Ritchie et al. 1985). Helicoverpa zea infestations that are concurrent with the time when soybean flowers can result in flower and pod feeding; dense infestations can cause severe crop loss (Eckel et al. 1992b). Flowering soybean plants support higher densities of H. zea larvae, purportedly because of the availability of multiple tissue types suitable for establishment and survival (Terry et al. 1989). When H. zea larvae establish on flowering soybean plants, later instar larvae feed on subsequently developing pods. Soybean plants are able to compensate for reproductive tissue loss resulting from feeding by shuffling photosynthetic assimilates into remaining reproductive structures when flowers are damaged. However, it is harder for soybean plants to compensate for loss of reproductive parts when they are in later maturity stages, especially if injury occurs late in the season or plants are compromised by other stress factors (McPherson & Moss 1989; Eckel et al. 1992b).

It is possible that flowering soybean plants can compensate for high densities of 4th instar H. zea larvae in certain geographies. For example, in locations where generations are distinct, rather than overlapping, the flower-feeding generational cohort can pupate in the soil allowing the soybean plant to compensate for loss before the next generational cohort starts feeding. A better understanding of the feeding preferences of H. zea may further clarify the relationship between feeding and soybean yield.

Helicoverpa zea larvae have been observed feeding on soybean leaves, stems, flowers, and pods in the field (Eckel et al. 1992a). Larvae can survive on soybean plants in any growth stage, but establishment success decreases as plants mature to pod-filling growth stages (Terry et al. 1989). In the field, H. zea populations generally establish, and are found, in the highest densities during the R1–R2 growth stages; they can reduce flower number through florivory, especially during the early stadia (Eckel et al. 1992a). Early instar larvae injure the calyx and ovaries on the flowers, whereas late instar larvae typically injure the entire flower (Terry et al. 1987a, 1989). Injured flowers may abort or remain in place, resulting in a reduced number of seeds per pod, subsequently reducing yield (Eckel et al. 1992b; Bi et al. 1994). Once larvae reach later instars, they feed on pods and seeds (Terry et al. 1987a; Herbert et al. 2003). Larvae perform best, as measured by pupal weight, when feeding on pod-filling plants, which suggests post-bloom plants may be a more suitable host for later instars (Terry et al. 1987a,b).

Most studies measure insect preference from a female oviposition perspective; however, experiments presented in this manuscript address preference from the perspective of larvae. Insect preference is defined as an encounter with host plant tissue types, including insect feeding behaviors, and is based on the assumption that current feeding preference is not influenced by previous host selection (Singer et al. 1992). Host plant tissues such as stems and leaves are generally not fed upon when flowers are present in the field. Because florivory is documented in the literature, we hypothesized that flower tissue was a preferred tissue type for feeding. However, because larvae develop in tandem with plant development, we also hypothesized that one host tissue type might not support the nutritional needs of larvae through all stadia, resulting in an individual larva feeding on multiple tissue types throughout its development. If this is true, larvae may prefer to feed on different tissues as early and later instars because of host tissue availability and nutritional quality. The objectives of this study were to determine the performance of larvae when feeding on different soybean tissues and to determine the feeding preferences when given a choice of soybean tissues.

**Materials and Methods**

We compared the performance of 2nd and 4th instar H. zea larvae when fed a single soybean tissue type, and the preference of these same instars when given a choice of a range of soybean tissue types. Performance was defined by survival to pupation when larvae were fed a single soybean tissue type. Preference was measured by feeding that was observed when larvae were given a choice of soybean tissue types.

A colony of H. zea was initiated with insects obtained from a laboratory colony maintained for 10 yr by the Corn Insect Host Plant Resistance Laboratory of the United States Department of Agriculture at Mississippi State University. The colony used in this study was reared on diet using parameters adapted from Wald Bauer et al. (1984) and held at 27 °C at a 16:8 h L:D photoperiod. Larvae used in experiments were chosen from the colony at random from available 2nd or 4th instar larvae (depending on the assay) and returned upon reaching pupation.

Soybean tissues, with the exception of flowers, were collected twice each week and stored in a sealed container with a moist paper towel at 10 °C for no more than 5 d. Because flowers senesced quickly after being removed from the plant, they were collected more frequently and closer to the date that they were used in the bioassays. Bioassay arenas were lined on the bottom with a 3% mixture of Apex™ Bioresearch Products Drosophila Agar Type II (Genesee Scientific Corporation, San Diego, California). Soybean plant tissues were placed on top of the agar for the experiments and included 1) expanding and 2) fully expanded trifoliates from the top two-thirds of the plant, 3) flowers (R1–R2), 4) immature pods (a mixture of R3–R4), 5) developed pods with developing seeds (R5), 6) pods with developed seeds (R6), 7) stems, and 8) petioles from the top two-thirds of the plant. The availability of each tissue type varied throughout the growing season; therefore, replications were timed based on host tissue type availability. A combination of greenhouse and field-grown soybean plants were used as sources of tissues. Source soybean plants were not treated with insecticides for the duration of their development and all soybean plants were determinate variety AG6130, maturity group 6 (Monsanto Company, St. Louis, Missouri). Greenhouse soybean plants were grown in 15.24 cm (6 inch) plastic pots using potting mix and were watered every other day.

**NO-CHOICE ASSAYS**

Tissues used in the no-choice assays included 1) fully expanded and 2) expanding trifoliates, 3) flowers (R1–R2), 4) stems, 5) petioles, 6) maturing pods (R3–R4), 7) developed pods with developing seeds (R5) and 8) developed pods with developed seeds (R6). No-choice assays of each single tissue type were conducted either in Petri dish (100 × 15 mm, Fisherbrand™ Polystyrene, Fisher Scientific Company LLC, Hampton, New Hampshire) or plastic container (7.6 cm height, 11.4 cm diameter, Berry Plastics Corporation, Evansville, Indiana) arenas with a single tissue type and a single insect larva. Disposable Petri dishes were used for the first 10 replications per tissue, but plastic container arenas were used for the remaining replications to reduce waste. Each combination of tissue type and instar was replicated 20 times. Plastic container arenas were cleaned with dish soap and hot water and dried fully before each use. Plant parts and arenas were changed every 2 d. During each replacement, insects were carefully moved from one arena to another using wide, round tip, featherweight forceps (BioQuip® Products, Inc., Rancho Dominguez, California) to minimize impact on the insect, and the larval stadium was recorded. Plant parts and insects were weighed at the beginning of each assay and at each replacement from the time the insect was placed in the assay until pupation or until...
the insect died. Similar amounts of soybean plant tissue were placed in the arenas at each replacement.

CHOICE ASSAYS

Tissues included in each choice assay were 1) emerging trifoliates, 2) fully emerged trifoliates, 3) flowers (R1–R2), 4) undeveloped pods (R3–R4), and 5) seed-filling pods (R5). Insects fed exclusively on stems and petioles in no-choice assays did not survive to pupation; therefore, these tissues were excluded from choice assays. Each assay was set up in a Petri dish arena (150 × 15 mm, Fisherbrand® Polystyrene) with a mixture of host soybean tissue types. Tissues were arranged around the circumference and either single 2nd or 4th instar larva, depending on the assay, was placed in the middle of the dish. Tissues were replaced every 2 d and weighed before and after replacement. Insects were weighed and larval stadia recorded every 2 d each time tissues were changed. Each choice assay was replicated 20 times. Feeding was recorded when tissues had visible feeding injury and frass around tissue. Feeding observations for a particular individual continued until pupation or death. Preference was determined by the number of times a particular tissue was fed on for each instar from the 2nd instar until pupation.

DATA ANALYSES

Arithmetic body weight, relative amount of food consumed, and relative growth rates were calculated for each assay by determining the amount of tissue consumed and the average weight over the entire life of each insect (Waldbauer 1968; Farrar et al. 1989). Relative growth rates were calculated using the formula

\[ RGR = \frac{G}{TA} \]

where \( G \) is the fresh weight gain of the insect during the feeding period, \( T \) is the duration of feeding period in days, and \( A \) is the mean fresh weight of the insect during the feeding period (Waldbauer 1968). Larval weights were recorded as the actual weight of the insect each time tissues were changed. Feeding observations were conducted every other day as tissue was replaced until pupation or death. Survivors were defined as individuals who reached the pupal stage, and development was considered complete when the individual reached pupation. The percentage of larvae reaching pupation in no-choice assays was calculated for each soybean host tissue type and instar (either 2nd or 4th). Tissue types with relatively low numbers of individuals reaching pupation \((n \leq 3)\) were omitted from the analyses.

Growth rates, pupal weights, relative amount of food consumed, larval weights, and days to pupation were each analyzed using a separate general linear mixed analysis of variance model (ANOVA, proc MIXED, SAS® software Version 9.3; SAS 2011). Larval weight was analyzed using repeated measures analysis, coded using the REPEATED statement in the SAS® software. The covariance structure was coded as compound symmetry. Pupal weight values were square root transformed to satisfy the assumptions of ANOVA, but the raw means are presented. The simple effect of tissue type was analyzed at each date and observed feeding in choice assays were included in analyses as binomial variables (insects either survived or fed or they did not) and were each analyzed using a generalized linear mixed ANOVA model for binomial data (proc GLIMMIX) in the SAS® software. For each analysis, insect, plant tissue source (field versus greenhouse grown), and date were modeled as random variables and tissue type was modeled as a fixed effect. Denominator degrees of freedom were calculated following the methods of Kenward & Roger (1997). If tests were significant, Tukey honestly significant differences (HSD) tests were used to determine differences among groups.

Results

NO-CHOICE ASSAYS

Second instars only survived to pupation on leaves and floral tissue. Survival rates were higher when 2nd instars fed newly emerging trifoliates and fully emerged trifoliates compared with flowers (Fig. 1; \( F = 7.05; \text{df} = 2,71; P = 0.0016 \)). There were no differences among newly emerged trifoliates, fully emerged trifoliates, and flowers for the total days required for 2nd instars to reach the pupal stage \( (F = 1.09; \text{df} = 2,10; P = 0.3732) \), for the arithmetic body weight means \( (F = 3.25; \text{df} = 7,3.91; P = 0.1393) \), for the relative amount of food consumed \( (F = 0.28; \text{df} = 3,12.3; P = 0.8362) \), or for the relative growth rates \( (F = 3.93; \text{df} = 7,1; P = 0.3707) \). Larval weights varied by different soybean tissue types over time \( (F = 22.18; \text{df} = 7,205; P < 0.0001) \) (Table 1).

Fourth instars survived to pupation on more tissue types than did 2nd instars, with the most survivors produced from fully developed pods with developed seeds (R6), followed by newly emerging and fully emerged trifoliates, followed by seed-filling pods (R5) (Fig. 2; \( F = 11.27; \text{df} = 4,19.1; P < 0.0001 \)). Trends were similar when pupal mass was measured, with the highest pupal mass obtained from larvae reared on fully developed pods with developed seeds (R6), followed by those reared on trifoliates, and then followed by those reared on developing pods (R4) (Fig. 3; \( F = 7.26; \text{df} = 5,88.3; P = 0.0001 \)). Moreover, when 4th instars developed on a particular tissue type, there were no significant differences for arithmetic body weight means \( (F = 1.70; \text{df} = 5,3.95; P = 0.3145) \), the relative amount of food consumed \( (F = 2.25; \text{df} = 5,9.34; P = 0.0016) \), or relative growth rates \( (F = 1.70; \text{df} = 5,3.95; P = 0.0016) \).

CHOICE ASSAYS

In choice assays, more feeding by 2nd instars was recorded on newly emerging trifoliates and developing pods (R4) than on other tissue types (Fig. 4; \( F = 7.94; \text{df} = 4,83.6; P < 0.0001 \)). Although early instars did not survive when fed solely on developing pods (R4), developing pods was one of the preferred tissue choices. Second instars did not survive to the pupal stage during the choice assays. Fifty-five percent of the larvae survived until 3rd instar and 20% until 4th instar. Fifteen percent of the individuals were killed by a fungus, likely introduced from field-collected tissues.

There was no significant difference in the proportion of feeding by 4th instars on different tissue types \( (F = 0.10; \text{df} = 4,60; P = 0.9831) \).

Fig. 1. Percentage of survivors (defined as individuals that reached the pupal stage) in no-choice assays after placement of Helicoverpa zea 2nd instars on a single soybean tissue type. Letters represent means separation by the Tukey HSD test \((α = 0.05)\) and error bars represent SE. Data marked by an asterisk (*) were omitted from the analysis because no individuals survived to pupation.
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Fourth instar survival rate to pupation was 90%. Feeding preference patterns changed with stadia. Whereas 2nd instars preferred to feed on emerged trifoliates, 4th instars preferred to feed on newly emerging trifoliates (R5) ($F = 2.03; df = 30,260.9; P = 0.0017$).

**Discussion**

Second instars of *H. zea* survived best on emerging and fully emerged soybean trifoliates, and soybean leaf tissue alone could provide all the nutrition needed for larval development from the 2nd instar to the pupal stage. Fourth instars survived best on fully developed pods with developing seeds (R6), based on no-choice assay results. Larval weights changed relative to the tissue type they fed on over time. These findings suggest that feeding behaviors may change as larvae mature, a hypothesis supported by observations in our choice assays. For instance, early instars did not perform well on undeveloped pods (R4), but they did feed on these tissues when given a choice over their larval lifespan. This could suggest that certain soybean tissues, such as developing pods, are a good food source but can only be exploited by older larvae. Hence, injury to this tissue type may occur during the later stages of an infestation by *H. zea*. Larvae may need a variety of tissue sources to obtain enough nutrients for survival to pupation (Scriber & Slansky 1981).

The survival of 2nd instars feeding on leaf tissue implies that *H. zea* larvae are able to develop on soybean plants both pre- and post-bloom because they can survive solely on leaf tissue for their entire larval lifespan. The contribution of soybean to overall *H. zea* population densities across the agroecosystem is unknown, but our results suggest that soybean can serve as an important developmental host when they are present in the environment. Additionally, 2nd instar survival on leaf tissue could be one explanation why later-planted soybean plants foster higher *H. zea* densities compared with earlier plantings. Because later-planted soybean plants have both emerging and fully emerged...
trifoliates later in the season (especially determinate varieties), larvae have access to higher-quality leaf tissue for a longer period of time. Although leaf feeding does not typically result in yield loss (Turnipspeed & Kogan 1976), larval populations may develop on leaves and move to other tissue types. Regardless, if plant and *H. zea* phenology are in sync (from the perspective of the insect) and if feeding preferences in laboratory assays mirror similar behaviors in the field, then early instars will feed on leaf tissue in the field. This could lead to populations composed of late instars, or infestations from consecutive generations, that feed on soybean reproductive tissue.

In the no-choice assays, *H. zea* larvae fed only floral tissue developed at the same rate as those that consumed other single tissue types. However, they did not survive as well when they consumed only flowers compared with consuming leaf tissue alone. Second instars, in particular, had a high mortality rate when they fed exclusively on flowers. This result could suggest that floral tissue lacks all necessary nutrients needed to complete larval development (Damle et al. 2005; McCall et al. 2013). However, these assays did not allow a perfect measurement of survival. It is possible that excised flowers differed in quality from those remaining on plants (Schmelz et al. 2003). Flowers were presented to the larvae soon after collection, but they quickly senesced and browned, presumably degrading some of the nutritive quality (Smith et al. 1992). Excising tissues can have a confounding effect on the quality of tissues, which was controlled by randomizing tissue samples in the assays. In the field, 2nd instar larvae could be avoiding predators by remaining inside flowers until they reach later instars. Our laboratory study was not designed to test these or other factors that must be explored in the field.

*Helicoverpa zea* larvae have been observed feeding on soybean leaves, stems, flowers, and pods in the field (Eckel et al. 1992a). Neonates and young larvae may feed on soybean trichomes and flowers if these tissue types are present (Mueller & Engroff 1980). Trichome erectness on soybean leaves has been shown to influence the abundance of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes abutiloneus* Haldeman (Hemiptera: Aleyrodidae) (Lambert et al. 1995), and trichome erectness, density, and sharpness can influence lepidopteran feeding behavior and survival (Turnipspeed 1977; Khan et al. 1986; Bhattacharyya & Ram 2001; Hulburt et al. 2004). Our assays did not test the effect of trichome density or shape on larval feeding, which could have affected larval feeding behaviors and survival (Turnipspeed 1977; Hulburt et al. 2004).

In the choice assays, 2nd instars fed more often on leaf tissue whereas 4th instars fed more often on pods and seeds. This preference could help explain why *H. zea* adult females prefer to oviposit in flowering soybean plants (Hardwick 1965; Johnson et al. 1975; Hillhouse & Pitre 1976; Eckel et al. 1992a), even though the larvae can fully develop using only leaf tissue. Because 2nd instar larvae can develop on leaves, flowers, and developing pods (R4), *H. zea* adults could prefer to oviposit in flowering soybean plants so that their offspring have optimal forage. As seen with flowers, 2nd instars did not survive when given only developing pods (R4), but they did feed on this tissue type in choice assays. Therefore, it is possible that developing pods can be used by larvae at some stage, but that they do not contain all the nutrients necessary for complete larval development (Scriber & Slansky 1981). Furthermore, fully developed pods with developing seeds (R6) provided the highest proportion of survivors with heavier pupae compared with other tissue types. Hence, adults produced from larvae feeding on fully developed pods with developing seeds (R6) will likely have high fitness and fecundity (Leuck & Perkins 1972; Honěk 1993).

The preference–performance hypothesis (Thompson 1988; Clark et al. 2011) states that female insects are expected to oviposit where feeding allows highest survival rates and best performance, but evidence in support of this mechanism of adult host selection for *H. zea* is mixed. Females of *H. zea* prefer to oviposit on flowering plants (Hardwick 1965; Johnson et al. 1975; Eckel et al. 1992a) and, in soybean, lay more eggs on flowering plants compared with other growth stages (Hillhouse & Pitre 1976). Furthermore, *H. zea* females prefer to oviposit on developing trifoliates in the top two-thirds of soybean plants (Terry et al. 1987b). This preference suggests that oviposition on later-planted soybean plants, which tend to flower during late July and early Aug in the southeastern USA, aligns with larval feeding preferences, assuming that the optimal foraging hypothesis (MacArthur & Pianka 1966) is true in this system. Whereas *H. zea* larvae are able to move within plants, female oviposition behavior has the potential to minimize foraging costs, assuming that energy expenditure for foraging is a major limiting factor (over others, such as predator avoidance, ease of site access for oviposition, etc.). However, in cotton that both does and does not express the insecticidal toxin produced by *Bacillus thuringiensis* (Bt), the preference–performance and optimal foraging hypotheses are not supported; *H. zea* females mainly oviposit on leaves or terminals and larvae are found mainly on flowers and small bolls (Farrar & Bradley 1985; Gore et al. 2002; Torres & Ruberson 2006). These tissues become available over time as the cohort of larvae and the plants develop together. Therefore, our results support both the preference–performance and optimal foraging hypotheses. Finally, assuming that *H. zea* is a major limiting factor in soybean production, our results justify promoting early planting dates, which would allow soybean crops to develop before *H. zea* immigrates from other crops in the agroecosystem.

Our findings have direct implications for traditional breeding as well as the development of transgenic varieties with insect resistance. *Helicoverpa zea* larvae have different survival rates on different tissue types and preferentially feed on various tissue types during different larval development stages. Expression of insecticidal toxins or plant morphological characteristics (i.e., leaf shape or trichome density) could potentially be modulated based on feeding preferences. Both transgenic cotton and corn differentially express toxins among tissue types (Olsen et al. 2005; Nguyen & Jehle 2007). To target 2nd instar larvae, it will be beneficial to have a higher expression of insect toxic compounds in leaf tissues especially if soybean is planted later in the season or double-cropped following wheat.

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