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Virus-Vector Relationship of Groundnut Bud Necrosis Virus Causing Mungbean Leaf curl Disease and its Vector *Thrips palmi* in Allahabad district of Uttar Pradesh

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ABSTRACT

Investigation on Virus- Vector relationship of Groundnut bud necrosis virus (GBNV) causing mungbean leafcurl and its vector *Thrips palmi* was carried out to study the acquisition access period, inoculation access period, incubation and retention period by serial transmission. Study showed that only larval stages with acquisition period of 2 days could acquire the virus and adults with inoculation period of two days could transmit. Minimum acquisition period for *T. palmi* adult to transmit the virus was found to be six hours, increase in inoculation access period increased transmission efficacy up to 48 hours. Inoculation access period of 72 hours showed reduced transmission (31.25 per cent) than 48 hours (35.84 per cent) Incubation period varied from 9-12 days after acquisition of GBNV by larvae, retention of GBNV by thrips varied from 7-16 days, pre-transmission intervals were in the range of 0-3 days.and transmission number varied from 3-13. Serial transmission studies showed that out of the 20 insects tested, 5 insects transmitted the virus throughout their life period.

Key words : Groundnut bud necrosis virus, Mungbean leaf curl, *Thrips palmi*, Virus – vector relationship

Mungbean leaf curl is one of the important diseases of mungbean which caused considerable loss in the past up to 40% in 33 districts of Uttar Pradesh as per the survey conducted by Nene (1972). Recent survey on the natural incidence and distribution of mung bean leaf curl in Allahabd district of Uttar Pradesh showed the incidence ranging between 1.88 to 49.76% (Kumar, 2007). The disease already assumed alarming level in southern states like Andhra Pradesh (Prasada rao *et. al*., 2003) and was reported to be transmitted by *T. palmi* in Andhra Pradesh (Sreekanth *et al*., 2006). As the disease is prevalent in Uttar Pradesh and causing considerable loss to mungbean, an investigation was done to establish the virus-vector relationship of mungbean leaf curl and *T. palmi* occurring in Uttar Pradesh.

MATERIAL AND METHODS

Collection, identification and rearing of Thrips Tender mungbean terminal leaves were collected from AAI-DU field during morning hours into a plastic jar. The jar was uncapped and covered with a glass funnel with a vial attached to the stem of funnel. Collected thrips in vials were immobilized by keeping them in a refrigerator for 15 minutes and were dislodged on to ice tray (Lewis, 1973). After cold treatment, immobilized thrips were identified and quickly separated into species using stereoscopic binocular microscope based on the key characters (Palmer *et al*., 1989; Palmer *et al*., 1990 and Reddy *et al*., 1991). Identified *T. palmi* was reared on detached cowpea leaflet method (Amin *et al*., 1981). After sex differentiation (Lewis,1973), *T. palmi* was cultured for virus-vector relationship as per Sreekanth *et al*.(2006), thrips from these cultures were used in further studies on virus-vector relationship.

Transmission studies with larvae and adults

Freshly emerged first instar larvae, from lab cultures of *T. palmi* were released gently on to the diseased mungbean leaf let portions floating on water in petridish and allowed for two days to acquire the virus. Larvae from the infected source were transferred to glass vials containing healthy greengram leaf lets. The vials were kept for incubation at 22-26^{0}C for adult emergence. After emergence, five adults were released on to 2 days old greengram seedlings in green house pot culture

and the seedlings were caged by plastic jars having thrips proof mesh at the top. Released thrips were given two days inoculation access period (IAP), later seedlings were sprayed with 0.025% methyl demeton to keep the plants free from further thrips damage. The seedlings were kept in glass house for further investigation. Freshly emerged larvae in large numbers were given two days acquisition access period (AAP) and were divided into three sets. Immediately after AAP, first set of larvae was given two days IAP, the second set of larvae was given IAP up to pupation and third set of larvae was allowed to become adults. Emerged adults were used in transmission tests after giving two days IAP. Adults were also tried for transmission with 2 day AAP and 2 days IAP. Five larvae or five adults per greengram plant were released so as to ensure transmission despite differential inoculum concentration acquiring and differential feed probing nature of thrips (Vijayalakshmi,1994), newly emerged adults which were not given AAP in the larval stage were given two days each of AAP and IAP. Five adults per plant were released.

Acquisition access period determination for *T. Palmi*

T. palmi first instar larvae were tested in AAP of 5, 10,15, 20, 25 30, 45 minutes, one hour, 6 hour, 12 hour, one day, two days and three days. The exposed larvae were transferred and allowed to become adults on healthy mungbean leaflets in an incubator at 22-26 ^{0}C. Five newly emerged adults were given 2 days IAP on to each mungbean plant.

Determination of inoculation access period and efffect of thrips number on transmission rate

T. palmi larvae after an AAP of two days were allowed to develop into adults and were given IAP of 5,10,15minutes,30minutes, 45 minutes,one hour,6hours,12hours,one day, two days and three days on mungbean seedlings. Five adults

Table1.Transmission studies of mungbean leaf curl virus with larvae and adult of *T. palmi* in mungbean, urdbean and cowpea hosts

Experiment	Test plant	No. of plants (5, Ist instar larvae or adults were released/plant)		Transmission (%)
		Tested	Infected	
Larvae with 2 day AAP and IAP	Mungbean (local variety)	81	0.00	0.00
	Urdbean(local)	77.00	0.00	0.00
	Cowpea(local)	74.00	0.00	0.00
Larvae with 2 day AAP day and IAP upto pupation	Mungbean (local)	88.00	0.00	0.00
	Urdbean(local)	82.00	0.00	0.00
	Cowpea(local)	70.00	0.00	0.00
Larvae with 2 day AAP and adult with 1 day IAP	Mungbean (local variety)	74.00	0.00	0.00
	Urdbean(local)	71.00	0.00	0.00
	Cowpea(local)	66.00	0.00	0.00
Larvae with 2 day AAP and adult 2 day IAP	Mungbean (local variety)	79.00	31.00	39.24
	Urdbean(local)	69.00	24.00	34.78
	Cowpea(local)	78.00	22.00	28.20
Adult 2 day AAP and 2 day IAP	Mungbean (local variety)	80.00	0.00	0.00
	Urdbean(local)	81.00	0.00	0.00
	Cowpea(local)	73.00	0.00	0.00

per mungbean plant were released for finding transmission efficiency. Adults of *T. palmi* were allowed two days IAP singly as well as in groups of 2,3,4,5,10 and 15 per plant. Number of infected plants were recorded based on symptoms.

Determination of incubation & retention period

Freshly emerged first instar larvae in sets of 15 each which are convenient for handling were given acquisition access period of two days were allowed to become adults on healthy mungbean leaf lets. Immediately after the adult emergence, a single viruliferous adult was transferred serially to each of the mungbean seedling at one day interval until its death. Experiment was conducted by maintaining twenty viruliferous adults through out. Incubation period was counted from the time of acquisition of the virus in the larval stage to the first transmission after adult emergence. Pre- transmission interval (interval between adult emergence and the first transmission), retention period (from the first transmission to the last transmission), post-transmission intervals (interval between last transmission and death), number of transmissions by individual viruliferous adult during its life were recorded. Transmission studies were conducted using mungbean, urdbean and cowpea as host plants.

RESULT AND DISCUSSION

Identification of thrip species

Based on the taxonomic keys (Palmer *et al*., 1989; Palmer *et al*., 1990 and Reddy *et al*., 1991), collected thrips were categorized into species. *T. palmi* was identified by straw yellow pale brown color, female size of 0.9mm long, pronotum with two pairs of setae on the posteriolateral margin, no setae on the anteriolateral margin, forewings with broken rows of wingvein setae and larvae whitish.

Transmission studies with larvae and adults

As per table.1, larvae with 2 day acquisition acess period (AAP) and adults with 2 day incoculation acess period (IAP) transmitted the virus in mungbean (39.24%), urdbean(34.78%) and cowpea (28.20%). Study showed that only larval stages could acquire the virus (AAP 2days) and transmitted by adults with incoculation access period of 2 days. Sreekanth *et al*. (2006) reported that adults of *T. palmi* could not acquire the virus,

Table 2. Influence of acquisition access period on transmission of mungbean leaf curl virus by *T. palmi*

AAP(I^{st} instar larvae were used)	No.of Plants (5 adults with an IAP of 2 days were released per plant)		Transmission (%)
	Tested	Infected	
5min.	38	0	0.00
10min.	44	2	4.54
15min.	42	3	7.14
20 min.	36	2	5.55
25 min.	40	3	7.5
30min.	38	6	15.78
45min.	44	7	15.90
1hr.	37	7	18.91
6hr.	36	8	22.22
12hr.	41	11	26.82
24hr.	43	16	37.20
48hr.	51	24	47.20
72hr.	32	9	28.1

Table 3. Influence of Inoculation Access Period on transmission of mungbean leaf curl virus by *T. palmi*

IAP (I[st] instar larvae with AAP of 2d were used)	No.of Plants (5 adults with an IAP of 2 days were released per plant)		Transmission (%)
	Tested	Infected	
5min.	54	0	0.00
10min.	49	0	0.00
15min.	53	0	0.00
20 min.	44	0	0.00
25 min.	38	0	0.00
30min.	35	0	0.00
45min.	51	0	0.00
1hr.	56	0	0.00
6hr.	48	6.00	12.50
12hr.	51	11	21.56
24hr.	44	12	27.20
48hr.	53	14	35.84
72hr.	48	13	31.25

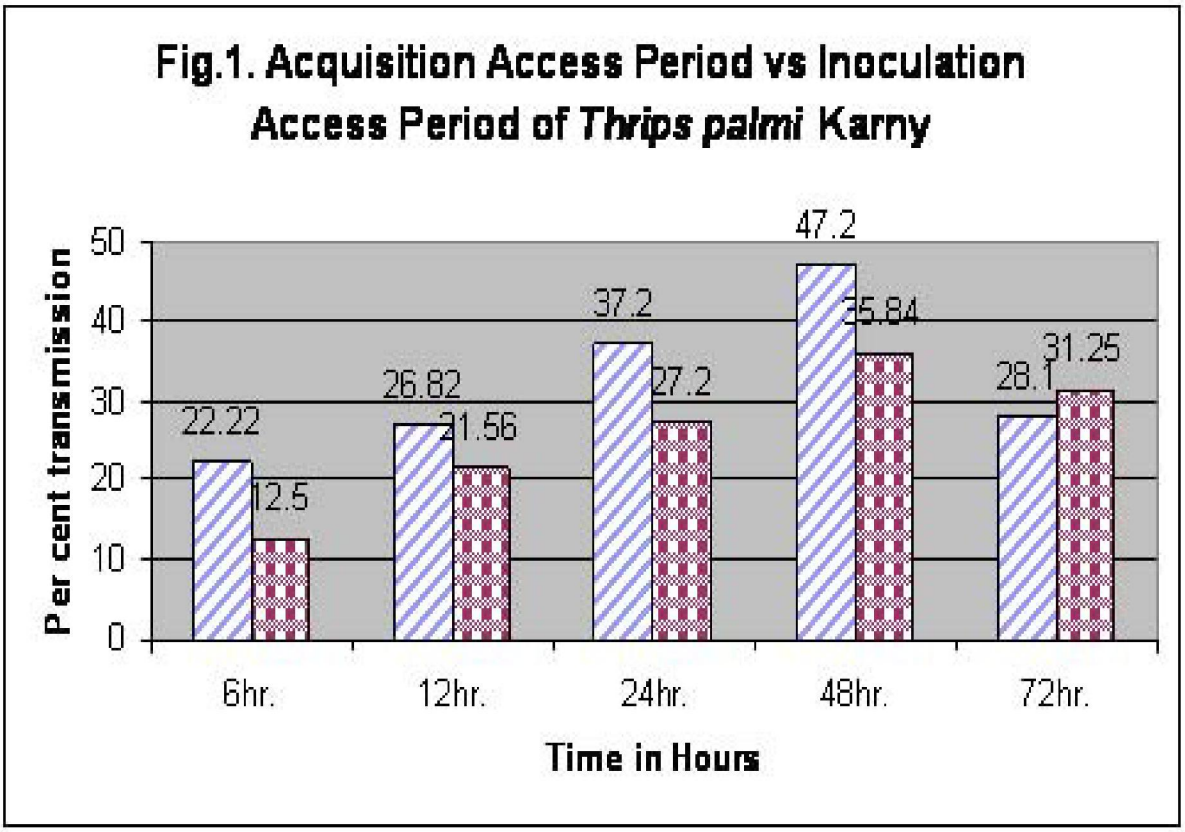

Table 4. Influence of no. of adult *T. palmi* population on transmission of mungbean leaf curl virus

T. palmi (adult/plant)	No. of attempts	No.of Plants		Transmission (%)
		Tested	Infected	
1	1	30	8	26.66
	2	30	9	30.00
2	1	30	10	33.33
	2	30	11	36.66
3	1	30	13	43.33
	2	30	12	40.00
4	1	30	14	46.66
	2	30	16	53.33
5	1	30	18	60.00
	2	30	19	63.33
10	1	30	30	100.00
	2	30	30	100.00
15	1	30	30	100.00
	2	30	30	100.00

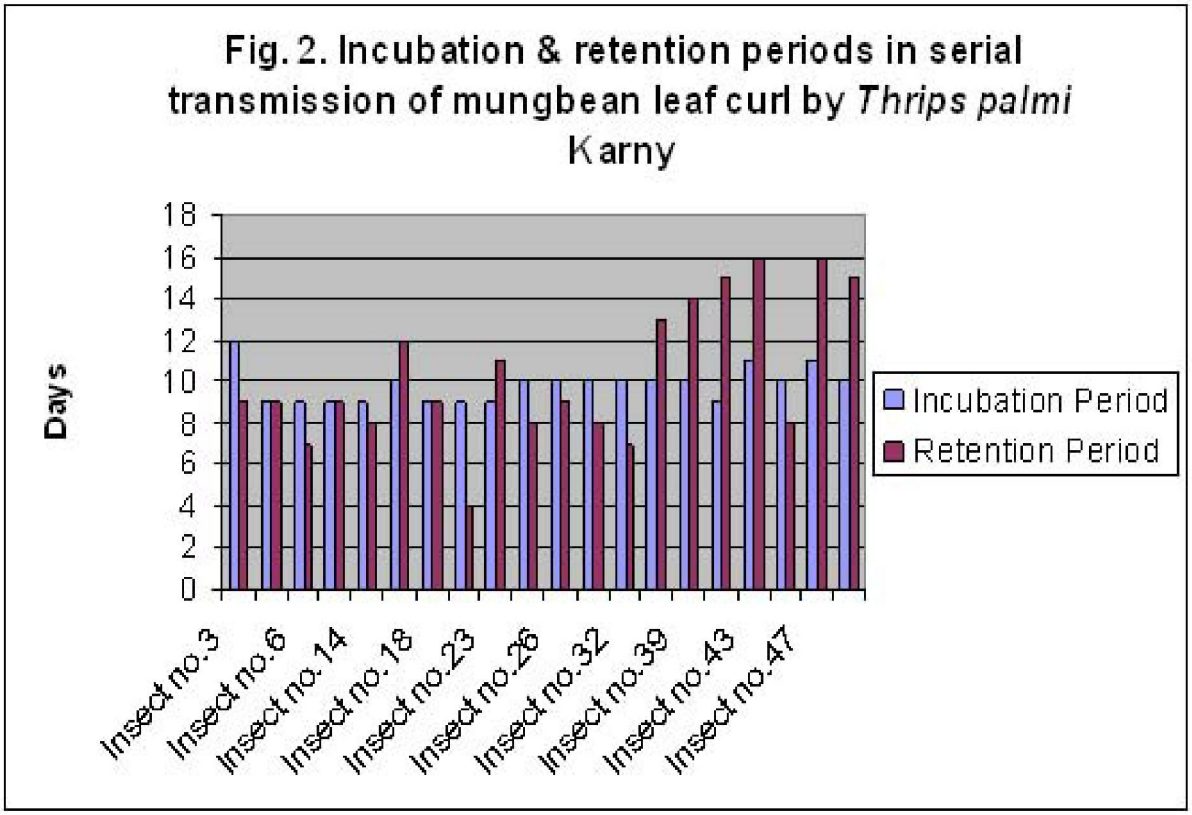

Table 5. Incubation and Retention periods as influenced by serial transmission of peanut bud necrosis virus by *T. palmi* after acquisition access for 2 days

Days from acquisition																						Incub[b] ation period (days)	Pre [c] Transm Ission Intervals (days)	Rete[d] ntion Period (days)	Post[e] trans Mission (days)	Active Trans Mission (days)	Trans mission no.
Transmission by individual *Thrips palmi* Karny [a]																											
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29						
Insect No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21						
3		E-	-	+	+	+	-	+	-	-	+	+	-	-	-	D-		-				12	3	9	4	69.20	6
5		E+	+	+	+	-	-	+	+	+	-	+	-	-	-	D-						9	0	9	4	73.30	8
6	E-	+	-	-	+	+	-	+	+	-	-	-	-	-	-	D-						9	0	7	7	53.30	5
8		E+	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-	D-				9	0	9	8	53.00	5
14		E+	+	-	+	+	+	-	+	-	D-											9	0	8	2	80.00	6
17		E-	+	+	-	+	-	-	-	-	-	-	+	+	D-							10	1	12	1	91.66	5
18		E+	+	+	-	-	-	+	-	+	-	-	-	D-								9	0	9	4	69.20	5
20		E+	+	+	+	D-																9	0	4	1	80.00	4
23	E-	+	-	+	+	-	-	+	+	-	-	D+										9	0	11	3	100.00	6
25		E-	+	-	+	+	+	+	+	+	-	-	-	-	-	-	D-					10	1	8	7	53.33	7
26	E-	-	+	+	+	+	+	+	-	-	D+											10	1	9	3	100.00	7
30		E-	+	+	-	-	-	-	-	+	-	-		-			D-					10	1	8	7	53.33	3
32		E-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	D-				10	1	7	9	43.75	3
33		E-	+	-	-	-	+	-	-	-	-	-	+	-	+	-	D-					10	1	13	2	87.70	4
39		E-	+	+	+	-	-	+	-	+	-	+	-	-	-	+	D-					10	1	14	1	93.33	7
41		E+	+	+	+	+	-	-	+	+	+	-	-	-	-	D+						9	0	15	5	100.00	10
43		E-	-	+	+	+	-	+	-	+	-	+	+	-	-	-	+	-	+	D+		11	2	16	0	100.00	10
45	E-	-	+	-	+	+	+	+	+	D+												10	2	8	0	100.00	7
47	E-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	-	D-	11	3	16	2	88.90	13
49	E-	-	+	+	+	+	-	-	+	+	-	-	-	-	-	+	-	+	-	D-		10	2	15	2	88.90	8

a-emerged adults were transferred individually to each mungbean seedlings serially; b- Period lapsed between acquisition of virus and the first transmission after becoming adult

c- Pre-transmission interval time lapsed between adult emergence and first transmission; d- Period lapsed between first transmission and last transmission e- Post- transmission interval time elapsed between the last transmission and death ; E- emergence of adult ; D- death; + / - indicate the transmission & non – transmission of virus.

while their larvae could acquire. Similar reports were made by Cho *et. al*. (1988); Palmer *et al*. (1990); Pappu *et al*. (1998) while working with thrip transmission of tomato spotted wilt virus and by Vijayalakshmi, (1994) on groundnut. In addition, Sakimura (1963) and Reddy *et al*. (1995a) reported that the ability to acquire tospovirus decreased with the age of the larva

Acquisition access period (AAP)

Minimum acquisition period for *T. palmi* larvae was 10 minutes (table.2). Virus was not transmitted at 5 minutes and thereon transmission gradually increased with increase in AAP from 10minutes (4.54% transmission), 15minutes (7.14 %), 20minutes (5.55 %), 25minutes (7.5%), 30 minutes (15.78%), 45 minutes (15.90%), 1hours (18.91%), 6hours (22.22%), 12hours (26.82 %),24hours(37.20%) up to 48hours (47.05%) and then decreased at 72 hours (28.2%) (Fig.2). Sreekanth *et al*. (2006) reported a minimum of 15 minutes acquisition access period (AAP) by larvae, Vijayalakshmi (1994) reported minimum acquisition access period of 5 minutes for *T. palmi*. The variation can be ascribed to the differential acquiring ability of vector or to the variability in virus.

Inoculation access period (IAP)

Minimum inoculation access period for *T. palmi* adult to transmit the virus was found to be 6 hours (12.5%) and the efficacy increased with increase in time at 12 hours (21.56%), 24 hours (27.2%) and 48 hours (27.08%). IAP for 72 hours showed reduced transmission (26.41%) than 48 hours (27.08%). IAP of 24 and 48 hours was the best treatment for effective transmission of GBNV by *T. palmi* (table.3). However, the inoculum concentration and number of punctures made as feeding attempts vary with feeding habit of individual insect as it is quite evident (table.4). Vijayalakshmi,(1994) reported 1 hr. IAP for *T. palmi* and the transmission of GBNV by *T. palmi* were found to increase with IAP up to 2 days (Vijayalakshmi *et al*., 1995), Sreekanth *et al*., (2006) reported 45 minutes inoculation access period (IAP) by adults was required for successful transmission. Inoculation access period (IAP) was found to be less for the studied *T. palmi*

Influence of adult *T. Palmi* number on GBNV transmission

With increase in no. of *T. palmi* , PBNV transmission per cent increased from 1 (26.66-29.99), 2 (33.33-36.66),3 (39.99-43.32), 4 (46.66-53.32), 5 (59.99-63.32),10 (100), 15 (100) (table.4).*T. palmi* abundance is positively correlated with GBNV transmission.These results are in corroboration with Vijayalakshmi (1994) report of 100 per cent transmission with 10 adults per groundnut seedlings. However, the results are differing with Somaraju and Subba rao (1993) report of minimum of three adult thrips required for successful transmission. It can be substantiated by the fact that transmission per cent depends on nature of vector probing to acquire or to inoculate virus. With increase in number of thrips, the number of punctures resulting from probing increases and thereby increasing transmission per cent which is in confirmity with Wijkamp and Peters (1993).

Determination of Incubation and Retention Period by serial transmission

Out of twenty *T. palmi* tested for their adult emergence only six thrips have emerged on 9^{th} day from acquisition and the rest on 10^{th} day after acquisition. None of the 9^{th} day emerged *T. palmi* transmitted virus on the day. On the 10^{th} day after acquisition eight thrips (no.5, 6, 8, 14, 18, 20, 23 and 41) transmitted GBNV. Insect No. 17,25,26,30,32,33,39,45 and 49 transmitted the GBNV on 11^{th} day after acquisition. Insects no.3,43,and 47 transmitted GBNV on 12^{h} day after acquisition. The emergence of adults varied from 9^{th} -10^{th} day after acquisition of virus.

Life span of the adult varied from 5 (No.20) - 21 days(No.47) (table.5). As per table-5 & fig.2, incubation period varied from 9-12 days after acquisition of GBNV by larvae, Vijayalakshmi (1994) reported incubation period of 7-16 days, Sreekant *et al*. (2006) reported incubation period of 10-12 days after acquisition of GBNV by larvae. The deviation between incubation periods may be

ascribed to acquisition of differential inoculum concentration by thrips. However, pre-transmission intervals of 0-3 days of present investigation is in conformity with Sreekanth *et al.* (2006).

Retention of GBNV by thrips in present study varied from 7-16 days, where as Vijayalakshmi (1994) reported minimum retention period as 2 days, which can be attributed to the differential probing for incoculum acquisition by thrips.The maximum retention period of present investigation was less (sixteen days) compared to twenty days of Vijayalakshmi (1994). Sreekant *et al*.(2006) reported that *T. palmi* remained infective for 2-18 days after insect becoming viruliferous and transmission number also varied from 3-13 compared to 2-15 of Sreekanth *et al.* (2006). Serial transmission studies showed that, of the 20 insects tested, 5 insects (23,26,41,43&45) transmitted the virus throughout their life period. Therefore, it is quite evident that the virus–vector relationship slightly differed for GBNV- *T. palmi* of South India and North India, However this differentiation can be attributed to the differential feeding behaviour of individual thrips species or virus variability.

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