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Repellent and Insecticidal Activity of Sequential Extracting Fractions Obtained from BPH-Resistant Rice Varieties against Brown Planthopper (*Nilaparvata lugens*)

Sung Eun Kim[1,3*], Young Doo Kim[2], Bo Kyoung Kim[1], Jae Kwon Ko[1] and Jae Chul Chun[3]

[1]*Honam Agricultural Research Institute, NICS, RDA, Iksan 570-080, Korea*
[2]*Yeongnam Agricultural Research Institute, NICS, RDA, Milyang 627-803, Korea*
[3]*Research Center of Bioactive Materials, Chonbuk National University, Chonju 561-756, Korea*

Abstract : Rice plant extracts of brown planthopper (BPH) resistant rice varieties, Jangseongbyeo (JSB) and Hwacheongbyeo (HCB) at different growth stages (seedling, tillering, heading and ripening) were sequentially fractioned using hexane, ethyl ether, ethyl acetate, butanol, and distilled water. The extracts were applied to BPH susceptible rice variety, Dongjinbyeo (DJB), to investigate the insecticidal and repellent effects against BPH. BPH insecticidal effects were not clearly observed with almost all of the extract fractions obtained from both JSB and HCB varieties for 12 h, whereas the ethyl ether and hexane extract fractions showed about 10 to 30% of BPH mortality in 24 to 48 h of application periods. An effective BPH repellent activity was found with the applications of ethyl ether extract fractions obtained from JSB variety. The extract fractions obtained from HCB variety did not show any different repellence among the various fractions. The BPH repellent effects of the extract fractions obtained at different growth stages of either JSB or HCB varieties did not show any correlations. The effect of ethyl ether fraction on BPH repellent was continually increased by 30 h after treatment and thereafter decreased. In addition, the first sub-fraction separated by a flash column chromatography eluted with chloroform:methanol (9:1, v/v) from the BPH effective ethyl ether fraction in JSB variety might be meaningful to repel BPH from BPH susceptible target rice plants. The results indicated that the ethyl ether fraction obtained from JSB was higher in repellent activity than in insecticidal activity, and suggesting that there might be specific substance(s) in the first sub-fraction (sF1) of the ethyl ether fraction in JSB that could provide repellent activity against BPH. (Received June 8, 2006; accepted June 20, 2006)

Key words : brown planthopper, BPH resistant rice variety, chemical extract fractions, insecticidal effect, repelling activity

INTRODUCTION

Brown planthopper (BPH, *Nilaparvata lugens*) is one of the most destructive insects in worldwide rice cultivation regions, especially in Asia (Shigematsu, *et al.*, 1982). Development of BPH resistant rice variety has been considered as one of approaches to avoid or reduce BPH damages. IR26 released in 1973 was the first rice variety with genetic resistance to BPH and emphasized the practical significance of varietal resistance as a basic measure (IRRI, 1975). However, the mechanism of varietal resistance in rice to BPH has remained unidentified.

BPH feeds by sucking sap from the phloem of rice (Sogawa, 1973). Sogawa and Pathak (1970) demonstrated that resistance to BPH in certain rice varieties is mainly governed by the presence of chemicals confined to the phloem tissues that inhibit feeding. Various chemical components have been extracted from rice varieties and have been reported to possess insecticidal and/or

*Correspounding author (Tel : +82-63-270-2548, E-mail : jcchun@chonbuk.ac.kr)

repelling activities against BPH. Petroleum ether extract fraction from some rice plants has been introduced to contain insecticidal substances and Zhang *et al.* (1999) reported that 3 nitraphthalic acid isolated from the ether fraction of a BPH resistant rice plant was an insecticidal and repellent substance against BPH.

Several researchers have interested in the repelling or antifeeding effect of specific chemicals from certain rice plant varieties to BPH. Various organic chemicals reported as repellents or antifeedants against BPH are trans aconitic acid (Koh *et al.*, 1976), soluble silicic acid (Yoshihara *et al.*, 1979), oxalic, maleic, and trans-aconitic acid (Yoshihara *et al*, 1980), and sterols such as β-sitosterol (Shigematsu *et al.*, 1982). However, any of those organic acids has not clearly provided a BPH-insecticidal, repellant or antifeedant effect with the separated single component. Therefore, we have assumed that a complex of substances in the specific fractions extracted by different extracting materials might effectively exert their activities against BPH.

This study was conducted to address the question of whether there is BPH varietal resistance in Korean rice varieties on the bases of presence of BPH resistance-related substance, to relate BPH resistance of the rice varieties with resistance-related substances, and to determine BPH insecticidal and repellant effects of the extract fractions on BPH susceptible rice variety.

MATERIALS AND METHODS

Rice Plants

Three rice varieties, Jangseongbyeo (JSB), Hwacheong-byeo (HCB), and Dongjinbyeo (DJB) were selected to compare their resistant responses to BPH. Seeds of the three rice varieties were sown in alternate row in a rectangular pot covered with nylon net and grown to 2 and 5 leaf stages. Ten third instar larvae of BPH were transferred into the cage. When the first dead plant caused by BPH damages was appeared, resistance responses were visually estimated with the injury rating from 0 to 9. In the injury rating, naught represents 'no injury', whereas nine stands for 'completely killed'.

Fractionation Procedure of Chemical Extracts from BPH Resistant Rice Cultivars

Four growth stages (seedling, tillering, heading and ripening stage) of JSB and HCB varieties were selected. Two kilograms of the rice plant tissues were ground and successively extracted with 75% methanol. The extract was filtered through Whatman No.1 filter paper and concentrated at 40°C using a vacuum evaporator. The concentrated extract was then sequentially fractioned with *n*-hexane, ethyl ether, ethyl acetate, buthanol and distilled water. Each fraction was concentrated to near dryness and the concentrated extract of the fractions was diluted with 60% acetone. The diluted extracts were used for examining *in vivo* bioassay. After the bioassay, the ethyl ether extract fraction was found to be an effective extract fraction against BPH. The extract fraction was then separated into different sub-fractions using a flash silica gel column chromatography set with a silica gel (70~230 mesh, 100 g, Merck) column (3.6×60 cm) eluting with chloroform:methanol (9:1, v/v) mixture. The eluted solution was collected in 10 mL each using a fraction collector. Each 10 mL of the solution was successively designated as 8 sub-fractions (sF1, sF2, sF3, sF4, sF5, sF6, sF7, and sF8). The sub-fractions were also in turn employed for *in vivo* bioassay.

In vivo screening for repellent effect against BPH

DJB, BPH susceptible rice cultivar, at seedling stage was transplanted in a circular pot. After untreated control plant was separated from target plants with a transparent cellophane paper to avoid contact of the extract fractions to the control plant, a proper concentration (10.0 g L^{-1}) of the extract obtained from JSB or HCB variety was treated on the target rice plant only. Twenty BPH were transferred onto the target rice plant. After removing the transparent cellophane paper, the pots were stored in a greenhouse at 25°C. The repellent effect against BPH was determined at various time intervals after treatment by counting the number of BPH remained on the target rice plants. Percent repellent effect was presented by subtracting percent BPH obtained with extract fraction treatments from percent BPH remained at the untreated control. There were three replications.

In vivo screening for insecticidal activity against BPH

DJB at seedling stage was transplanted in a circular pot and twenty BPH were transferred into the pot. A proper concentration (5.0 g L^{-1}) of extracts obtained from two different rice varieties, JSB and HCB, were directly treated on the BPH using a hand pumped plastic sprayer connected with 50 mL spray glass bottle until the BPH was wet enough. After the pots were stored in a greenhouse at 25°C for 12, 24, and 48 h, the insecticidal effect against BPH was timely measured. The experiment was replicated three times.

RESULTS AND DISCUSSION

Differential resistance to BPH

Three rice varieties responded differently against BPH (Table 1). Among the rice varieties tested, DJB at 2 leaf stage was the most susceptible to BPH. The susceptibility was not changed with the growth stage, so that similar injury rating was also recorded at 5 leaf stage of DJB. On the contrary, almost no injury by BPH was observed with JSB at both growth stages studied. However, damages found in HCB were remained in between those of DJB and JSB. These results indicated that JSB and HCB were strongly resistant and moderately resistant to BPH, respectively, whereas DJB was susceptible to BPH. Based on the results, DJB was employed as BPH bioassay test plant in further *in vivo* screening study.

Table 1. Different resistance responses of three rice varieties against BPH at different growth stages

Rice variety	Injury rating (0 – 9)[1]	
	2 leaf stage	5 leaf stage
Jangseongbyeo	1	0
Hwacheongbyeo	5	4
Dongjinbyeo	9	9

1Injury rating: 0 = no injury, 9 = completely killed.

Repellent Activity

BPH repellent activity of various extract fractions obtained from JSB and HCB at different growth stages is presented in Figure 1. Out of five fractions extracted on the basis of different solvent polarities, the ethyl ether fraction obtained from JSB showed the highest repellent activity against BPH, whereas ethyl acetate, butanol, and distilled water fractions gave rise to rather negative repellent effects. The negative effect might possibly indicate BPH attraction by any substances in the fractions. Moreover, the repellent activity was not greatly changed as the rice variety grew. Unlike JSB, however, all the fractions from HCB did not show any repellent activity. Although both hexane and ethyl ether fractions of HCB provided positive repellent activity, the effect was not high enough to compare those of JSB. These results clearly indicated that JSB was more resistant to BPH than HCB and the resistant difference might be possibly due to certain substances which exert repellent activity against BPH. In particular, the repellent activities of the ethyl ether fractions obtained from JSB were consistent throughout all the growth stages, suggesting that active resistance related substances might not be produced as the rice plant grows, but be originally presented in the plant body.

Dose dependent BPH repellent activity of the ethyl ether fraction was evaluated with different application times (Fig. 2). The repellent effect against BPH using the ethyl ether fraction increased continually as increasing the application time by 30 h, regardless of the application concentrations and thereafter the effect decreased at 42 h. In addition, increase in application concentration of the fraction resulted in almost proportional increase in the repellent effect. The fact that the repellent effect was affected by both application concentration and time strongly suggests presence of potential active substance(s) in the ethyl ether fraction.

The ethyl ether fraction of JSB was further divided into eight sub-fractions using chloroform:methanol solvent system and their BPH repellent activities were presented in Figure 3. Among eight sub-fractions only the first sub-fraction (sF1) provided effective repellent activity against BPH. The effect was recorded by about 27% repellent effect at 3 h after treatment and increased to about 45% at 18 h. However, the effect was gradually decreased with time. On the other hand, little or no repellent effect was obtained with the rest of sub-fractions, sF2 to sF8.

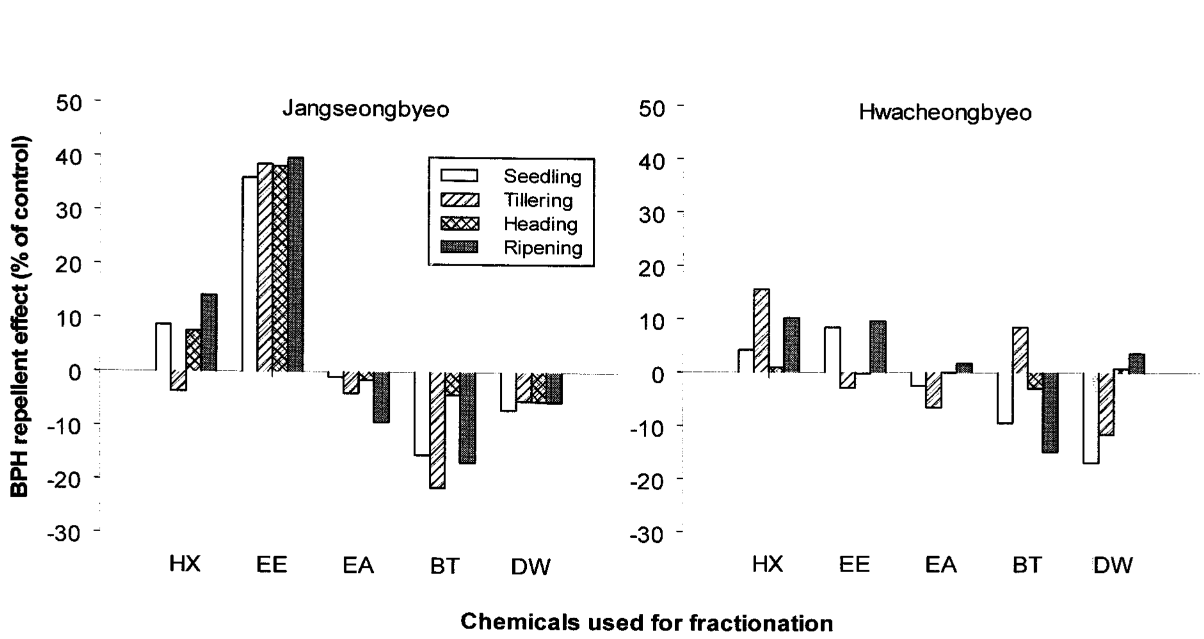

Fig. 1. BPH repellent effect of the extract fractions obtained from BPH resistant rice varieties at different growth stages: HX, hexane; EE, ethyl ether; EA, ethyl acetate; BT, butanol; DW, distilled water. The repellent effect was presented as subtracting the effect of untreated control from results of the extract fractions.

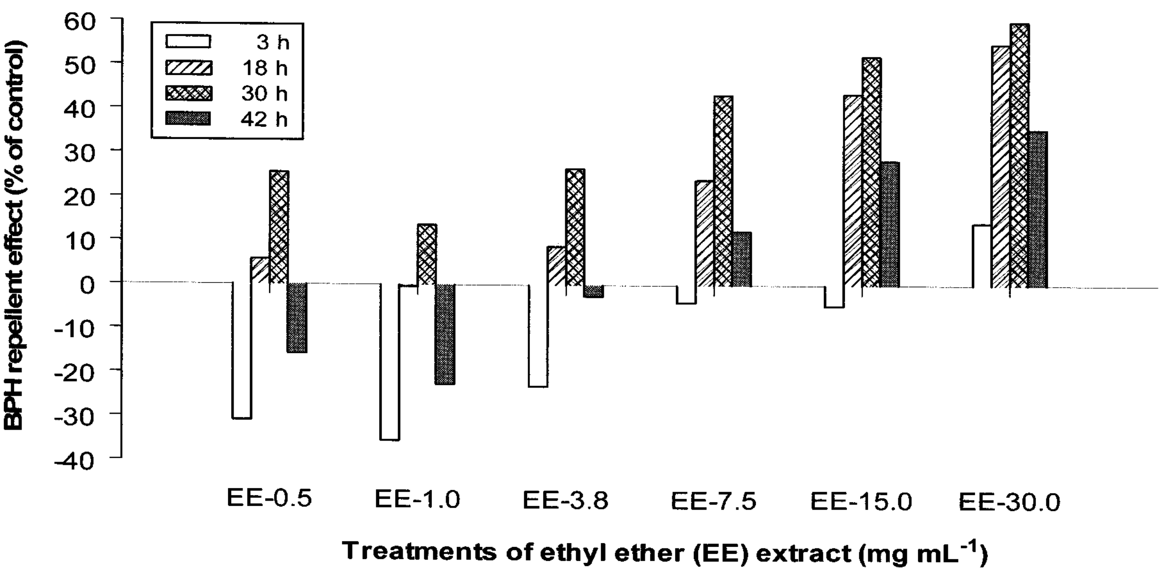

Fig. 2. BPH repellent effects as influenced by the treatments of ethyl ether extract fractions in different concentrations and application periods.

Insecticidal Activity

BPH mortality due to JSB and HCB extracts obtained at different growth stages (seedling, tillering, heading and ripening stages) is presented in Table 1. The methanol extracts obtained from JSB and HCB at various growth stages were fractioned due to the polarity of different extractants such as hexane, ethyl ether, ethyl acetate, buthanol and distilled water. The concentration of extract fractions applied to BPH was 5.0 kg L^{-1}. At the fixed concentration of extracts, insecticidal activity against BPH was not obtained with most of the extract fractions applied. Only about 17 to

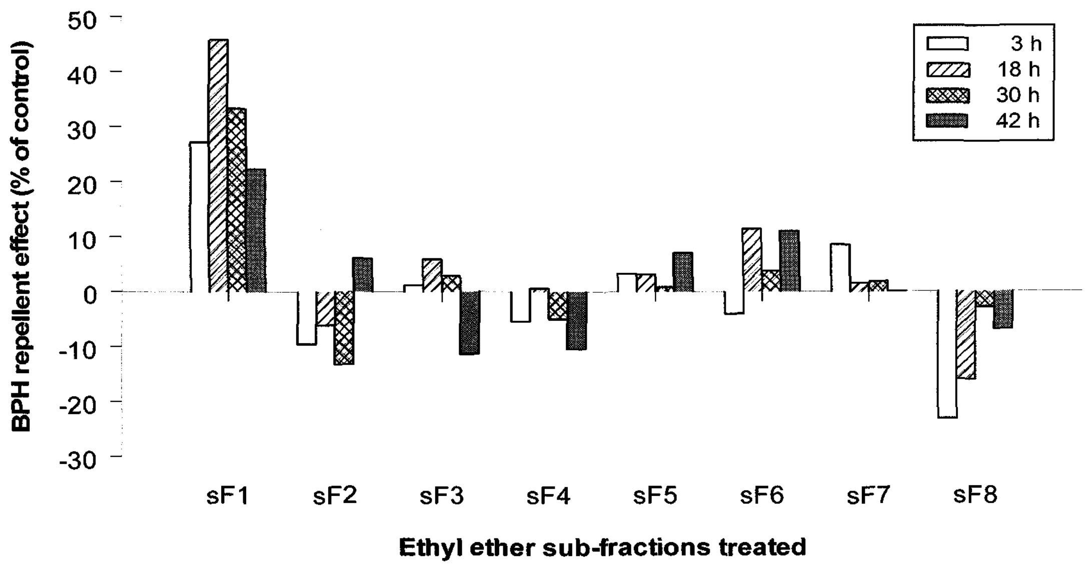

Fig. 3. BPH repellent effects of the sub fractions in the ethyl ether extract fraction obtained from Jangseongbyeo variety : sF1 sF8, subfraction 1 to 8.

33% insecticidal effects against BPH were observed with ethyl ether and hexane extracts fractioned from JSB. With HCB, however, 10 to 20% BPH mortality was found as applied only with the hexane fractions. The insecticidal effects of both JSB and HCB fractions were not varied with fractions obtained at different growth stages.

Insecticidal effects of the rice extracts against BPH with different application periods (12, 24, and 48 h) are shown in Table 2. Insecticidal effects of the extract fractions were observed as similar trends presented above. The ethyl ether and hexane extract fractions of JSB activated only 20 to 33% BPH mortality in 24 and 48 h of application periods. Using the HCB extracts, 13 to 20% of the insecticidal effects were observed only with hexane fraction in the same application periods. However, the mortality of BPH as applied with all of the extract fractions was not observed within 12 h.

In contrast, Zhang *et al.* (1999) reported that petroleum ether extracts from resistant and susceptible rice varieties were significantly toxic to BPH, causing 98% and 73.5% mortalities, respectively and identified

Table 2. BPH mortality as influenced by the chemical extract fractions obtained from different growth stages of BPH resistant rice varieties

Source Rice Plants of Extracts	Chemical Fraction	Mortality (%)			
		Seedling	Tillering	Heading	Ripening
Jangseongbyeo	Untreated control	0	0	0	0
	Hexane	20	17	23	20
	Ethyl ether	27	30	30	33
	Ethyl acetate	0	0	0	0
	Butanol	0	0	0	0
	Distilled water	0	0	0	0
Hwacheongbyeo	Untreated check	0	0	0	0
	Hexane	13	20	10	13
	Ethyl ether	0	0	0	0
	Ethyl acetate	0	0	0	0
	Butanol	0	0	0	0
	Distilled water	0	0	0	0

Table 3. BPH mortality as influenced by the different application periods of chemical extract fractions

Source Rice Plants of Extracts	Chemical Fraction	Mortality (%)		
		12 h	24 h	48 h
Jangseongbyeo	Hexane	0	23	20
	Ethyl ether	0	30	33
	Ethyl acetate	0	0	0
	Butanol	0	0	0
	Distilled water	0	0	0
Hwacheongbyeo	Hexane	0	13	20
	Ethyl ether	0	0	0
	Ethyl acetate	0	0	0
	Butanol	0	0	0
	Distilled water	0	0	0

3-nitraphthalic acid as an active component. Therefore, the effects of specific chemical extracts from rice plant varieties against BPH might be dependent upon the characteristics of the source plants that provide specific extracts. In this study, the ethyl ether and hexane fractions extracted from the BPH resistant rice varieties, JSB and HCB, were not expectable to use as insecticidal chemicals even though insecticide effect was observed with those fractions because considerably high concentrations of the extract fractions might be necessary to activate as an insecticidal substance against BPH.

In conclusions, in this study we selected BPH resistant rice varieties; JSB and HCB, to find specific rice plant extract fractions effective as BPH insecticidal and/or repellent activities, since JSB and HCB varieties have shown a marked and relatively high resistance, respectively, to BPH as comparing with DJB that is one of the most BPH susceptible variety. With the treatments of all extract fractions (hexane, ethyl ether, ethyl acetate, butanol, and distilled water) obtained from both the BPH resistant varieties did not show any insecticidal activity against BPH with 12 h. However, the ethyl ether and hexane extract fractions in JSB and/or HCB varieties provides about 10 to 30% of BPH mortality in 24 and 48 h of application periods. In addition, the highest BPH repellent activity was also observed with the treatments of ethyl ether fractions that obtained from JSB plant variety. No specific repellent effect was found with other fractions from JSB and with all fractions extracted from HCB variety either. The repellent effects of the various extract fractions depending on the different growth stages of both JSB and HCB varieties were not closely correlated. The effect of ethyl ether fraction on BPH repellent was continually increased for 30 h, and then it was decreased. In particular, the first sub-fraction of ethyl ether fraction might be effective to repel BPH from the target rice plant. Therefore, in future study, we need to identify the specific sub-fraction obtained from the ethyl ether extract fraction in JSB variety because the unknown substance(s) in the sub-fraction might be very useful as a natural repellent against BPH.

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벼멸구 저항성벼 품종 추출분획물의 기피 및 살충 활성

김성은[1,3]*, 김영두[2], 김보경[1], 고재권[1], 전재철[3]([1]*호남농업연구소*, [2]*영남농업연구소*, [3]*전북대학교 생리활성물질 연구소*)

요약 : 벼멸구 저항성벼 품종인 장성벼와 화청벼로부터 생육시기(유묘기, 분얼기, 출수기, 등숙기)별로 추출 용액 극성에 따라 얻은 추출분획물(hexane, ethyl ether, ethyl acetate, butanol 및 water)에 대하여 벼멸구 살충 및 기피 활성을 벼멸구 감수성벼 품종인 동진벼를 검정 대상식물로서 조사하였다. 장성벼와 화청벼로부터 얻은 추출분획물 대부분은 12시간 처리 기간 중 벼멸구 살충 활성을 보이지 않았으나, ethyl ether 및 hexane 추출분획물은 24시간 및 48시간 처리에서 약 10~30%의 벼멸구 살충 활성을 나타내었다. 한편 장성벼의 ethyl ether 추출분획물은 효과적인 벼멸구 기피 활성을 보였지만, 화청벼로부터 얻은 추출분획물은 이러한 효과를 나타내지 않았다. 장성벼 또는 화청벼의 여러 생육 단계별로 얻은 추출분획물 사이에는 벼멸구에 대한 기피 활성 효과에 있어 뚜렷한 관련성을 찾을 수 없었다. Ethyl ether 분획의 벼멸구에 대한 기피 활성은 처리 후 30시간까지 증가되었으나, 그 이후에는 감소되는 경향이었다. 더욱이 장성벼 ethyl ether 추출분획물에 대한 flash column chromatography의 chloroform:methanol (9:1, v/v) 용출에 의한 8개의 2차 분획물(sF1~sF8) 중 최초의 분획물(sF1)은 벼멸구에 대해서 비교적 높은 기피 활성을 나타내었다. 이상의 결과는 장성벼 ethyl ether 추출분획물은 벼멸구에 대한 살충 활성 보다는 오히려 기피 활성이 높은 것으로 나타났으며, 이 ethyl ether 추출분획물의 2차 분획물들에 대한 검정 결과 최초의 2차 분획물(sF1) 중에는 기피 활성에 관여하는 물질의 존재 가능성이 매우 높은 것으로 나타났다.

색인어 : 벼멸구, 벼멸구 저항성벼 품종, 추출분획물, 살충효과, 기피효과

*연락저자 (Tel : +82-63-270-2548, E-mail : jcchun@chonbuk.ac.kr)