# TIME-MORTALITY FOR FRUIT FLIES (DIPTERA: TEPHRITIDAE) EXPOSED TO INSECTICIDES IN LABORATORY

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### ABSTRACT

Laboratory trials were conducted to measure the mortalities caused by six organophosphate and two pyrethroid insecticides against *Ceratitis capitata* (Wied.) and *Anastrepha fraterculus* (Wied.) adults under two systems: cover sprays and toxic baits. Differences were detected in terms of susceptibility for both fruit fly species to the insecticides. Deltamethrin and fenpropathrin sprayed on the insects presented the lowest lethal times  $(LT_{50})$  for *C. capitata* ( $\leq 5.2 \text{ min}$ ). Among the organophosphates, ethion and malathion were the most toxic to this species ( $LT_{50} \leq 10.2$ ). Fenpropathrin and malathion showed the lowest values of  $LT_{50}$  for *A. fraterculus* females (3.3 and 4.7 min, respectively) under cover spray. For *C. capitata* females treated with toxic baits, fenpropathrin and trichlorfon showed the lowest  $LT_{50} < 5.0 \text{ min}$ ) and chlorpyrifos presented the highest  $LT_{50}$  (65.7 min). For *A. fraterculus* females, trichlorfon and dimethoate showed the lowest  $LT_{50}$  (7.9 and 8.8 min, respectively). *Ceratitis capitata* was more tolerant to malathion, chlorpyrifos and dimethoate than *A. fraterculus*.

KEY WORDS: Insecta, Ceratitis capitata, Anastrepha fraterculus, mortality.

## RESUMO

TEMPOS LETAIS DE MOSCAS-DAS-FRUTAS (DIPTERA: TEPHRITIDAE) EXPOSTAS A INSE-TICIDAS EM LABORATÓRIO. Ensaios de laboratório foram conduzidos para medir as os tempos para causar mortalidades a adultos de *Ceratitis capitata* (Wied.) e *Anastrepha fraterculus* (Wied.) submetidos a seis inseticidas organofosforados e dois piretróides, sob duas formas: pulverização tópica e iscas tóxicas. Foram detectadas diferenças em termos de susceptibilidade de ambas as espécies de moscas-das-frutas aos inseticidas testados. Na forma de aplicação tópica, deltametrina e fenpropatrina apresentaram os menores tempos letais (TL<sub>50s</sub>) para *C. capitata* ( $\leq$  5,2 min). Entre os organofosforados, etiom e malatiom são os mais tóxicos para essa espécie. Fenpropatrina e malatiom demonstraram os menores valores de TL<sub>50</sub> para fêmeas de *A. fraterculus* (3,3 min e 4,7 min, respectivamente) na forma de aplicação tópica. Para fêmeas de *C. capitata* tratadas com iscas tóxicas, fenpropatrina e triclorfom mostraram os menores TL<sub>50</sub> (< 5,0 min) e clorpirifós apresentou o maior TL<sub>50</sub> (65,7 min). Para fêmeas de *A. fraterculus*, triclorfom e dimetoato mostraram os menores TL<sub>50s</sub> (7,9 e 8,8 min, respectivamente). *Ceratitis capitata* foi mais tolerante ao malatiom, clorpirifós e dimetoato que *A. fraterculus*.

PALAVRAS-CHAVE: Insecta, Ceratitis capitata, Anastrepha fraterculus, mortalidade.

## INTRODUCTION

Infestations of fruit flies (Diptera: Tephritidae) have caused losses in many fruit crops and imposed limits on the export market (RAGA et al., 2004). The quarantine regulations imposed by an importing country can either deny a producing country a potential export market, or force the producer to carry out an expensive disinfestation treatment against fruit flies (WHITE & ELSON-HARRIS, 1994).

Among 94 Anastrepha species reported in Brazil, the South American fruit fly, Anastrepha fraterculus

(Wied.), is the most important fruit fly, infesting 67 commercial and no commercial host species (ZUCCHI, 2000). In Brazil, 59 host fruits are registered for the exotic medfly *Ceratitis capitata*(Wied.), belonging to 21 botanical families (ZUCCHI, 2001).

Because fruit-fly females lay eggs beneath the exocarp of the fruit and the larva develop inside the fruit, there is a little chance of a pesticide affecting the larva (MORENO et al., 1994), especially for mature ones. Consequently, flies are the targets of the chemical control. Eight organophosphates and 2 pyrethroids are available for controlling fruit-fly populations in

Brazil for use in aerial spray and toxic bait (RAGA & SATO, 2005).

Currently, the most common Integrated Pest Management (IPM) strategy in Latin America to manage tephritids consists in the use of a bait spray (CALKINS & MALAVASI, 1995; MORENO & MANGAN, 2002), a mixture of an insecticide and an attractant, usually molasses or sugar cane syrup. In crops, such as stone fruits, mango, passion fruit and apple, cover sprays are used to control the flies.

Although insecticide resistance is not a serious problem for *C. capitata* (KOREN et al., 1984; WOOD & HARRIS, 1989), limited information about the susceptibility of other Tephritidae species to the pesticides is available in the literature. Thus, the objective of the present study was to evaluate the performance of some registered insecticides to control fruit flies in Brazil, in two application systems under laboratory conditions.

#### MATERIAL AND METHODS

### Colonies

*C. capitata* and *A. fraterculus* were obtained from the colonies maintained since 1993 at Instituto Biológico, in Campinas, SP, Brazil. Medfly larvae were reared in artificial media (RAGA et al., 1996) and South American fruit-fly larvae were reared in papaya fruits (RAGA et al., 1993). Flies received water and a 1:3 mixture of yeast extract and refined cane sugar after the emergence.

### **Cover spray assay**

Five females and five males of 1-2d-old *C. capitata* and 4-7d-old *A. fraterculus* were placed in each of 10 plastic Petri dishes (8.5 cm diameter). Each dish corresponded to one replication. About 2.0 ml of insecticide suspension was applied under a Potter spray tower at 60.0 kPa. The following insecticides at recommended doses (g AI/100 L of water) were tested: deltamethrin (1.25), ethion (100.0), chlorpyrifos (96.0), malathion (200.0), trichlorfon (150.0), fenthion (50.0), fenpropathrin (12.0) and dimethoate (200.0). Prior to the spraying, flies were stored in the refrigerator at about 1° C for 5 minutes. After the treatment, flies were maintained at room temperature and ambient humidity. Evaluations of survivorship were conducted at 5, 10, 15, 20, 25, 30, 35 and 40 minutes after initial exposure.

#### Toxic bait assay

Five females and five males of 2-4d-old *C. capitata* and 4-5d-old *A. fraterculus* were placed in each of 10

replicate small cages (1,400 cc). The commercial protein Bio Anastrepha at 5% was provided to the control population and it was added to all before mentioned insecticides at the respective doses. The pH of insecticide solutions ranged from 8.57 to 8.63. About 1.0 mL of bait was disposed through the cotton inside 2.7 cm plastic dishes. Dishes were placed on the cage ground. About 12h before the beginning of the experiments, flies were deprived of food and water. During the tests, only baits were available to the flies. Tests were carried out in the laboratory at 25  $\pm$  1° C, 70  $\pm$  10% RH and 14h photophase. The adult survival was registered at 15, 30, 45, 60, 75, 90, 120, 150, 180 and 195 min after initial exposure.

#### Statistical analysis

Irreversible knockdown followed by death of the adults was the criterion to determine mortality. The  $LT_{50}$  values for each compound were estimated using Probit analysis (Polo PC).

#### **RESULTS AND DISCUSSION**

#### **Cover spray assay**

Deltamethrin and fenpropathrin presented the lowest  $LT_{50}$  for medfly  $\leq 5.2$  min). Among the organophosphates, ethion and malathion were the most toxic to *C. capitata* adults ( $LT_{50} \leq 10.2$  min). The highest  $LT_{50}$  values were obtained with dimethoate ( $LT_{50} = 16.2$  min) for medfly females and for chorpyrifos ( $LT_{50} = 15.9$  min) in medfly males (Table 1). In terms of susceptibility no differences were detected between medfly female and male.

KOREN et al. (1984) observed that medfly males were significantly more susceptible than the females when exposed to malathion. These authors concluded that after 9 generations of medfly exposure to malathion in laboratory conditions, females can develop a slight resistance to this compound, and that this property is sex-limited, i.e. males were unable to develop any resistance.

Fenpropathrin and malathion application resulted in the lowest values of  $LT_{50}$  for *A. fraterculus* females (3.3 and 4.7 min, respectively) and males (5.1 and 4.7 min, respectively). Except for trichlorphon, no differences were observed between *A. fraterculus* females and males. South American fruit-fly females were more tolerant to trichlorfon ( $LT_{50} = 16.3$  min). Ethion reached the highest values of  $LT_{50}$  for *A. fraterculus* males (16.3 min). A maximum of 40 minutes provided 100% mortality for both sex of *C. capitata* and *A. fraterculus*.

Treatment	Species	Sex	LT <sub>50</sub> (min)	Slope ± SE	$X^2$	df
Deltamethrin	Af	Females	5.25 (4.26 - 6.08)	$4.37 \pm 0.55$	0.87	2
	Cc	Females	3.89 (2.35 - 5.13)	$2.70\pm0.21$	1.74	3
	Af	Males	5.18 (4.13 - 6.04)	$4.17\pm0.65$	1.61	2
	Cc	Males	2.87 (1.20 - 4.25)	$2.32 \pm 0.22$	1.83	3
Ethion	Af	Females	15.9 (13.0 - 20.4)	$1.88 \pm 0.21$	1.12	3
	Cc	Females	7.58 (5.88 - 9.08)	$2.45\pm0.11$	8.47	5
	Af	Males	16.3 (14.9 – 17.6)	$5.42 \pm 0.83$	4.10	2
	Cc	Males	9.26 (7.07 - 11.2)	$2.01\pm0.12$	5.97	4
Chlorpyrifos	Af	Females	13.2 (11.8 - 14.4)	$5.43 \pm 0.86$	4.92	2
	Cc	Females	13.5 (11.6 – 15.5)	$2.50\pm0.11$	9.08	5
	Af	Males	10.7 (9.36 - 12.01)	$3.66 \pm 0.27$	1.63	3
	Cc	Males	15.9 (14.1 – 17.6)	$3.87 \pm 0.16$	9.43	5
Malathion	Af	Females	4.67 (3.56 - 5.53)	$4.13 \pm 0.55$	0.46	2
	Cc	Females	10.2 (8.96 - 11.4)	$3.87 \pm 0.19$	6.67	3
	Af	Males	4.68 (3.48 - 5.64)	$3.66 \pm 0.87$	3.43	2
	Cc	Males	8.49 (7.31 - 9.60)	$3.79\pm0.16$	5.89	4
Trichlorfon	Af	Females	16.3 (13.3 – 22.7)	$2.14\pm0.33$	1.43	2
	Cc	Females	13.2 (11.5 – 14.8)	$3.01\pm0.12$	9.34	5
	Af	Males	9.38 (8.30 - 10.4)	$4.59\pm0.61$	4.17	2
	Cc	Males	14.6 (12.8 - 16.2)	$4.02\pm0.19$	5.42	4
Fenthion	Af	Females	7.59 (5.68 - 9.21)	$2.26\pm0.15$	0.48	3
	Cc	Females	14.0 (12.2 - 15.8)	$2.90\pm0.14$	6.96	4
	Af	Males	6.28 (4.83 - 7.58)	$2.74\pm0.52$	4.89	3
	Cc	Males	13.3 (11.8 – 14.6)	$4.92\pm0.22$	5.10	4
Fenpropathrin	Af	Females	3.29 (2.01 - 5.60)	$0.93 \pm 0.27$	1.42	3
	Cc	Females	5.20 (3.93 - 6.26)	$3.32\pm0.21$	1.25	3
	Af	Males	5.07 (3.94 - 6.01)	$3.77\pm0.75$	1.98	2
	Cc	Males	3.69 (2.16 - 4.91)	$2.73 \pm 0.22$	1.06	3
Dimethoate	Af	Females	7.91 (5.20 – 10.1)	$1.61\pm0.28$	3.73	4
	Cc	Females	16.2 (14.8 - 17.4)	$5.30\pm0.19$	4.81	5
	Af	Males	6.58 (4.64 - 8.26)	$2.07\pm0.47$	9.11	4
	Cc	Males	15.0 (13.6 - 16.3)	$5.14 \pm 0.27$	2.42	3

Table 1 – Comparison of lethal times ( $LT_{50}$ ) obtained for both sex of *Anastrepha fraterculus* (Af) and *Ceratitis capitata* (Cc) exposed to 8 insecticides under a Potter tower.

Hsu et al. (2004) suggest that the Oriental fruit fly *Bactrocera dorsalis* (Hendel) can become resistant to various insecticides, including malathion. Cross-resistance between chemicals may also be found for these species. KEISER et al. (1973) observed that *C. capitata* was 10 times more susceptible to malathion than *B. dorsalis* and *Bactrocera cucurbitae* (Coquillett) in topical application. Scoz et al. (2004) reached 100% mortality of *A. fraterculus* after exposing flies to fenthion and trichlorphon by residual contact in laboratory.

## **Toxic bait assay**

For medfly females, fenpropathrin and trichlorfon had the lowest  $LT_{50}$  values ( $\leq 5.0$  min), suggesting that they were more toxic than the remaining insecticides. Chlorpyrifos presented the highest  $LT_{50}$  (65.7 min) for *C. capitata* males (Table 2). Only chlorpyrifos and dimethoate showed differences of lethal times between medfly females and males. For both compounds females were more susceptible than males.

For *A. fraterculus* females, trichlorfon and dimethoate had the lowest  $LT_{50s}$  (7.9 and 8.8 min, respectively). The highest value (71.1 min) was obtained with ethion for *A. fraterculus* females. No differences in susceptibility between *A. fraterculus* females and males were detected among the compounds.

*Ceratitis capitata* wasmore tolerant to malathion, chlorpyrifos and dimethoate than *A. fraterculus*. In contrast, the South American fruit-fly was more tolerant to deltamethrin, ethion, trichlorfon, ethion, trichlorfon, fenthion and fenpropathrin than the medfly.

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Table 2 – Comparison of lethal times ( $LT_{50}$ ) obtained for both sexes of *Anastrepha fraterculus* (Af) and *Ceratitis capitata* (Cc) exposed to 8 toxic baits in laboratory cages.

Treatment	Species	Sex	LT <sub>50</sub> (min)	Slope ± SE	$X^2$	df
Deltamethrin	Af	Females	49.3 (42.8 - 55.7)	$2.70\pm0.26$	8.07	7
	Cc	Females	9.33 (4.51 - 13.2)	$2.56\pm0.23$	0.97	3
	Af	Males	47.4 (41.2 - 53.5)	$2.78\pm0.20$	4.24	7
	Cc	Males	6.81 (1.52 - 11.1)	$2.41\pm0.33$	1.12	2
Ethion	Af	Females	71.1 (64.8 - 78.3)	$4.18\pm0.34$	7.45	4
	Cc	Females	49.4 (43.2 - 55.7)	$2.85\pm0.09$	11.9	6
	Af	Males	61.5 (55.0 - 68.0)	$3.26\pm0.32$	10.93	6
	Cc	Males	44.2 (39.0 - 49.5)	$3.31 \pm 0.11$	9.01	5
Chlorpyrifos	Af	Females	17.5 (11.6 - 22.9)	$1.88\pm0.26$	5.23	6
	Cc	Females	50.1 (45.1 - 55.3)	$3.93\pm0.13$	8.51	5
	Af	Males	17.3 (12.1 – 22.1)	$2.19\pm0.30$	5.32	5
	Cc	Males	65.7 (59.8 - 71.7)	$4.38\pm0.14$	6.87	5
Malathion	Af	Females	9.68 (4.15 - 15.5)	$1.42\pm0.29$	7.32	6
	Cc	Females	37.9 (33.0 - 42.8)	$3.13 \pm 0.11$	3.66	5
	Af	Males	7.33 (2.92 - 11.9)	$1.80\pm0.24$	1.70	5
	Cc	Males	33.5 (28.2 - 38.7)	$2.61\pm0.10$	5.45	5
Trichlorfon	Af	Females	7.93 (5.38 - 9.44)	$5.41 \pm 0.96$	0.36	2
	Cc	Females	$\leq 5$	-	-	-
	Af	Males	7.66 (5.05 - 9.28)	$5.02\pm0.74$	0.59	2
	Cc	Males	$\leq 5$	-	-	-
Fenthion	Af	Females	29.3 (25.0 - 33.4)	$3.36\pm0.37$	6.38	5
	Cc	Females	19.8 (15.7 - 23.5)	$3.02\pm0.18$	2.01	3
	Af	Males	25.1 (21.9 - 28.2)	$4.59\pm0.54$	2.37	2
	Cc	Males	23.3 (19.6 - 26.6)	$3.56\pm0.19$	2.31	3
Fenpropathrin	Af	Females	35.7 (29.1 - 41.9)	$2.21\pm0.31$	9.91	6
	Cc	Females	$\leq 5$	-	-	-
	Af	Males	28.9 (24.1 - 33.4)	$2.91\pm0.23$	4.81	6
	Cc	Males	$\leq 5$	-	-	-
Dimethoate	Af	Females	8.79 (4.29 - 12.6)	$2.67\pm0.71$	3.60	2
	Cc	Females	20.6 (17.4 - 23.5)	$3.95\pm0.21$	1.22	3
	Af	Males	5.33 (0.84 - 9.85)	$2.15\pm0.60$	1.09	2
	Cc	Males	33.3 (27.7 - 37.7)	$4.21 \pm 0.27$	4.84	3

Since the 1950s, malathion-bait spray has been considered standard in fruit-fly eradication programs around the world (STEINER et al., 1961; HART et al., 1967; TROETSCHLER, 1983; KOREN et al., 1984; PECK & MCQUATE, 2000; VILLASEÑOR et al., 2000; SEEWOORUTHUN et al., 2000). Scoz et al. (2004) observed 100% mortality of *A. fraterculus* 24h after exposing the flies to fenthion and trichlorphon baits in the laboratory. The higher tolerance of medfly to malathion compared to that observed for the South American fruit fly is a promising aspect for some countries such as Argentina, Peru and Brazil, where medfly eradication programs are currently underway. The use of malathion baits against *C. capitata* can also control *A. fraterculus*.

The results of mortalities of *C. capitata* and *A. fraterculus* caused by insecticides under both application techniques suggest that we need to evaluate the adequate choice of active ingredients to

suppress natural populations in each fruit crop and IPM strategy. Further studies in field conditions are necessary to evaluate the period of control, doses and impact on non-target organisms.

#### References

- CALKINS, C.O. & MALAVASI, A. Biology and control of fruit flies (*Anastrepha*) in tropical and temperate fruits. *Revista Brasileira de Fruticultura*, v.17, p.36-45, 1995. Suplemento.
- HART, W.G.; INGLE, S.; REED, D.; FLITTERS, N. Bioassays of Mexican fruit flies to determine residual effectiveness of Mediterranean fruit fly bait sprays in Southern Texas. *Journal of Economic Entomology*, v.60, n.5, p.1264-1265, 1967.
- Hsu, J.; FENG, H; Wu, W. Resistance and synergistic effects of insecticides in *Bactrocera dorsalis* (Diptera:

Tephritidae) in Taiwan. *Journal of Economic Entomology*, v.97, n.5, p.1682-1688, 2004.

- KEISER, I.; KOBAYASHI, R.M.; SCHNEIDER, E.L.; TOMIKAWA, I. Laboratory assessment of 73 insecticides against the Oriental fruit fly, Melon fly, and Mediterrranean fruit fly. *Journal of Economic Entomology*, v.66, n.4, p.837-839, 1973.
- KOREN, B.; YAWETZ, A.; PERRY, A.S. Biochemical properties characterizing the development of tolerance to malathion in *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Journal of Economic Entomology*, v.77, n.4, p.864-867, 1984.
- MORENO, D.; MARTINEZ, A.J; RIVIELLO, M.S. Cyromazine effects on the reproduction of *Anastrepha ludens* (Diptera: Tephritidae) in the laboratory and in the field. *Journal* of Economic Entomology, v.87, n.1, p.202-211, 1994.
- MORENO, D. & MANGAN, R.L. A bait matrix for novel toxicants for use in control of fruit flies (Diptera: Tephritidae). In: Hallmann, G. & Schwalbe, C.P. (Eds.). *Invasive arthropods in agriculture*. Enfield: Science Publishers, 2002. p.333-362.
- Peck, S.L. & McQuate, G.T. Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera: Tephritidae) populations. *Journal of Economic Entomology*, v.93, n.2, p.280-289, 2000.
- RAGA, A.; SATO, M.E.; POTENZA, M.R.; GIORDANO, R.B.P.; SZULAK, C.; SUPLICY FILHO, N. Uso da radiação gama para desinfestação de mangas em relação a larvas de *Ceratitis capitata* (Wied., 1824), Anastrepha fraterculus (Wied., 1830) e Anastrepha obliqua (Macquart, 1835). *Ecossistema*, v.18, p.45-55, 1993.
- RAGA, A.; YASUOKA, S.T.; AMORIM, E.O.; SATO, M.E.; SUPLICY FILHO, N.; FARIA, J.T. Sensibilidade de ovos de *Ceratitis capitata* (Wied., 1824) irradiados em dieta artificial e em frutos de manga (*Mangiferaindica* L.). *ScientiaAgricola*, v.53, n.1, p.114-118, 1996.
- RAGA, A.; PRESTES, D.A.O.; SOUZA FILHO, M.F.; SATO, M.E.; SILOTO, R.C.; GUIMARÃES, J.A.; ZUCCHI, R.A. Fruit fly (Diptera: Tephritoidea) infestation in citrus in the State of São Paulo, Brazil. *Neotropical Entomology*, v.33, n.1, p.85-89, 2004.
- RAGA, A. & SATO, M.E. Effect of spinosad bait against *Ceratitis capitata* (Wied.) and *Anastrepha fraterculus* (Wied.)

(Diptera: Tephritidae) in laboratory. *Neotropical Entomology*, v.34, n.5, p.815-822, 2005.

- Scoz, P.L.; BOTTON, M.; GARCIA, M.S. Controle químico de Anastrepha fraterculus (Wied.) (Diptera: Tephritidae) em laboratório. *Ciência Rural*, v.34, n.6, p.1689-1694, 2004.
- SEEWOORUTHUN, S.I; P ERMALLOO, S.; G UNGAH, B.; S OONNOO, A.R.; ALLECK, M. Eradication of an exotic fruit fly from Mauritius. In: TAN, K. (Ed.). Area-wide control of fruit flies and other insect pests. Penang: Penebirt Universiti Sains Malaysia, 2000. p.389-394.
- STEINER, L.F.; ROHWER, G.G.; AYERS, E.L.; CHRISTENSON, L.D. The role of attractants in the recent Mediterranean fruit fly eradication program in Florida. *Journal of Economic Entomology*, v.54, n.1, p.30-35, 1961.
- TROETSCHLER, R.G. Effects on nontarget arthropods of malathion bait sprays used in California to eradicate Mediterranean fruit fly, *Ceratitis capitata. Environmental Entomology*, v.12, p.1816-1822, 1983.
- VILLASEÑOR, A.; CARRILLO, J.; ZAVALA, J.; LIRA, C.; REYES, J. Current progress in the medfly program Mexico-Guatemala. In: TAN, K. (Ed.). Area-wide control of fruit flies and other insect pests. Penang: Penebirt Universiti Sains Malaysia, 2000. p.361-368.
- WHITE, I.M. & EISON-HARRIS, M.M. Fruit flies of economic significance: their identification and bionomics. Wallingford: CAB International, 1994.601p.
- WOOD. R.J., & HARRIS, D.J. Genetics: Ceratitis capitata artificial and natural selection. In: ROBINSON, A.S. & HOOPER, G. (Eds.). Fruit flies: their biology, natural enemies and control. Amsterdam: Elsevier, 1989. v.3B, p.19-31.
- Zucchi, R.A. Espécies de *Anastrepha*, sinonímias, plantas hospedeiras e parasitóides. In: MALAVASI, A. & Zucchi, R.A. (Ed.). *Moscas-das-frutas de importância econômica no Brasil – conhecimento básico e aplicado*. Ribeirão Preto: Holos Editora, 2000. p.41-48.
- Zucchi, R.A. Mosca-do-mediterrâneo, *Ceratitis capitata* (Diptera: Tephritidae). In: Vilela, E.F.; Zucchi, R.A.; Cantor, F. (Eds.). *Pragas introduzidas no Brasil*. Ribeirão Preto: Holos Editora, 2001. p.15-22.

Received on 19/12/05 Accepted on 3/2/06