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Publisher's version available at:
http://dx.doi.org/10.1603/0046-225X(2008)37[719:AEOCPB]2.0.CO;2

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Activity Evaluation of Cocoa Pod Borer Sex Pheromone in Cacao Fields

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Environ. Entomol. 37(3): 719–724 (2008)

ABSTRACT The previously identified female sex pheromone of cocoa pod borer, *Conopomorpha cramerella*, was re-evaluated for its attractive activity in different field conditions. It was found that lures containing 100-μg of synthetic sex pheromone blend, (*E,Z,Z*)- and (*E,E,Z*)-4,6,10-hexadecatrienyl acetates, and the corresponding alcohols in a ratio of 40:60:4:6 in a polyethylene vial attracted male *C. cramerella* moths in Sabah and peninsular Malaysia and in Sumatra and Sulawesi, Indonesia, suggesting that the same pheromone strain existed in a wide stretch of the Indo-Malayan archipelago. Of the three kinds of trap designs tested, the Delta traps were more effective than Pherocon V scale traps. Male captures were not significantly different among traps baited with 100-, 300-, or 1,000-μg doses of sex pheromone. A release rate study of pheromone formulation conducted in the laboratory showed that volatile active ingredients were desorbed from polyethylene vials following first-order kinetics, which indicates a satisfactory "half-life time" of a 100-μg loading is $\approx$6 wk under laboratory conditions. A satisfactory attractiveness of the lure with a 100-μg loading was $\approx$1–2 mo in the fields.

KEY WORDS *Conopomorpha cramerella*, (*E,Z,Z*)- and (*E,E,Z*)-4,6,10-hexadecatrienyl acetates, release rate, field activity, *Theobroma cacao*

The cocoa pod borer, *Conopomorpha cramerella* (Snellen) (Lepidoptera: Gracillariidae), has been reported as the most serious pest of cacao, *Theobroma cacao* L., in Southeast Asia (Lim et al. 1982, Mumford 1986, Mumford and Ho 1988), and losses can be in excess of 30% of the crop (Beevor et al. 1986a). The management of *C. cramerella* has heavily relied on the applications of pesticides (Wood et al. 1992, Beevor et al. 1993). The sex pheromone components of *C. cramerella* were identified in 1986 (Beevor et al. 1986a, b, Ho et al. 1987) and field tested in Sabah, Malaysia. A mass trapping trial using sex pheromone was shown to reduce *C. cramerella* infestation in large-scale pilot studies (>200 ha) (Beevor et al. 1993), and a mating disruption trial was shown to reduce mating of females (Tay and Sim 1989, Alias et al. 2004). However, the use of pheromones against *C. cramerella* was halted in the early 1990s, partly because of economic reasons and partly because of lack of commercial quantities of pheromone preparations available for large-scale use. The failure of the previous attempts to manage *C. cramerella* using sex pheromone was also attributed to the possibility of existence of more than one strain of *C. cramerella* in Asia that behaved differently to the pheromone blend (Beevor et al. 1993, Matlick 1998). This would make it much harder to use species- or strain-specific pheromones in an integrated pest management (IPM) strategy.

Research on mate-seeking behavior of male *C. cramerella* that improves management tactics is therefore warranted. The availability of a sex pheromone and trapping system provides on opportunity to detect and monitor the infestation level of *C. cramerella.* Therefore, a new initiative was started in 2004, and the objectives of this study were to (1) re-evaluate the attractiveness of the *C. cramerella* sex pheromone in both Sabah and peninsular Malaysia and (2) determine whether the same or different pheromone strains of *C. cramerella* existed. This article describes

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field trapping studies using the *C. cramerella* sex pheromone in different geographic locations and in cacao fields in Malaysia and Indonesia.

Materials and Methods

Lure Fomulation. Pheromone blends from two sources were used in the field trials. One blend was synthesized and formulated at the Natural Resources Institute (NRI), Greenwich, United Kingdom, as described by Beevor et al. (1986a). The polyethylene vials (26 by 8 by 1.5 mm thick; Just Plastic, Norwich, United Kingdom) were impregnated with 100 μg of the synthetic sex pheromone blend of (*E,Z,Z*)- and (*E,E,Z*)-4,6,10-hexadecatrienyl acetates and the corresponding alcohols in a ratio of 40:60:4:6, and an equal weight of 2,6-di-*tert*-butyl-4-methylphenol (BHT) as antioxidant. We refer to this blend as NRI. The second pheromone blend was synthesized at Pest Control, Bangalore, India, and we refer to this blend as PCIL. The (*E,Z,Z*)-isomer of 4,6,10-hexadecatrienyl acetate and corresponding alcohol were prepared following the procedure of Beevor et al. (1986a), and the (*E,E,Z*)-isomers were prepared by stereochemical control of the Wittig olefin reaction (Anderson and Henrick 1975). The pheromones were formulated in Beltsville, MD, using three kinds of polyethylene vials. The above vials from the United Kingdom were loaded with 100, 300, and 1,000 μg of pheromone, whereas two kinds of vials from India (24 by 9 by 1.5 mm thick; Mic & Mac Plastic Industries, India) (INa and INb) were loaded with 100 μg of pheromone.

Field Trials. Each cacao field in this study was separated several kilometers away unless otherwise specified. The polyethylene vials were secured to Pherocon V scale traps at the manufactured lure insertion holes or suspended at the center of the interior of Delta and Pherocon 1C traps with 22-gauge wires. Traps in all experimental sites were hung from poles fixed on suitable tree trunks by strings and suspended at ≈0.5 m above the top of the tree canopy (≈4.5 m above ground level) (Ho et al. 1987). Treatments were arranged in a randomized complete block design in each site and ≈12-m intertrap distance. Traps were checked weekly unless otherwise specified, liners were replaced when the accumulated numbers of moths exceeded 150 per trap or as necessary, and the treatments were rotated among positions after checking within replicate.

For the trap comparison study, three kinds of traps (Delta, Pherocon V scale, and Pherocon 1C; Trécé, Salinas, CA) were evaluated in three cacao fields at the Cocoa Research and Development Center, Malaysian Cocoa Board (MCB) in Sabah, Malaysia, using the NRI 100-μg pheromone formulation. Six traps of each type were tested, and traps were checked daily. The Delta trap (Trécé) baited with the NRI 100-μg pheromone formulation was used at all sites for the rest of studies in Malaysia.

For the pheromone activity evaluation study in Malaysia, two treatments (three pheromones and three blank controls) were tested in four cacao fields at Cocoa Research and Development Center in Sabah and two cacao fields in the Cocoa Research and Development Center in peninsular. Two treatments (four pheromones and four blank controls) were tested in two adjacent (500 m) commercial fields in Teck Guan Estate, Sabah.

The Delta traps (Pest Control) were used for trapping at all sites in the Indonesia. For lure longevity study, five pheromone lures (NRI 100-μg pheromone formulation) were tested out in 12 plots at Rambong Sialang Estate, Sumatra. For the lure comparison study, six kinds of pheromone formulations (United Kingdom vials loaded with 100, 300, and 1,000 μg of India pheromone; two kinds of India vials, INa and INb, loaded with 100 μg of India pheromone; and NRI 100-μg pheromone formulation) were evaluated at two cacao fields with no pesticides applied in Noling (Yaminas), Sulawesi. For the pheromone activity evaluation study, two treatments (four traps baited with 100 μg of Pest Control pheromone formulation and four blank controls) were tested in four cacao fields in the Effem Foods Southeast Asia-PT Effem in Sulawesi.

Lure Release Rate. A release rate study was conducted in the Beltsville laboratory for 4 mo. Twelve sex pheromone lures (NRI 100-μg formulation) were suspended on hooks in a fume hood (temperature: 20–25°C, face velocity: 129 ft/min). Three lures were removed from the fume hood at the first day of each moth and stored in a freezer at −20°C until analysis. Three new lures were used as day 0 standards. Each lure was placed in a glass vial with a cap, and 1 ml of hexane was added. After soaking for 48 h at room temperature, the hexane solution of each sample was analyzed by gas chromatography-mass spectrometry (GC-MS). Electron impact GC-MS analyses (70 eV) of pheromone lures were conducted on a Hewlett–Packard 6890 GC coupled to a HP 5973 Mass Selective Detector using a DB-WAXETR capillary column (J&W Scientific, Folsom, CA; 60 m by 0.25 mm ID, 0.25-μm film thickness, temperature programmed at 150°C for 2 min and then to 230°C at 15°C/min and held for 15 min) with helium as the carrier gas. The ions m/z 79 and 107 were selected as monitor ions, and the pheromone concentrations remaining were calculated by comparison with the new lure standards.

Data Analyses. Residual amounts of pheromone determined from vials were analyzed using regression analysis. Pheromone activity evaluation data were analyzed using χ^2 goodness-of-fit tests. The trap comparison data were converted to proportions of male moth catch and transformated to arcsine $\sqrt{p}$, where p is the original proportion, to stabilize the variance. Means were compared by one-way analysis of variance (ANOVA) followed by the Ryan-Einot-Gabriel-Welsch range test (SPSS 10.0 for Windows; George and Mallery 2002) for significance at $\alpha = 0.05$. The dose–response data were analyzed using R statistical software (R Development Core Team 2006) lme4 add-on package (Bates and Sarkar 2007). A generalized liner mixed model based on a quasipoisson distribution (to accommodate overdispersion) and a log

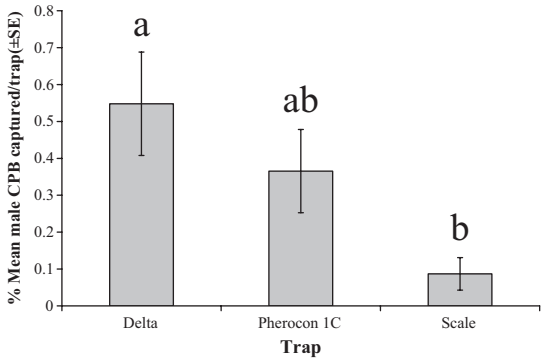

Fig. 1. Mean proportions of total male *C. cramerella* (±SE) caught in traps (Trécé) baited with synthetic sex pheromone blend (NRI) at the Cocoa Research and Development Center, Sabah, Malaysia.

link was used (McCulloch and Searle 2001). The random effect was date by location combination (48 groups). An overall test on the effect of the six treatments was made using a likelihood-ratio test (against a model that did not differentiate among the treatments; χ^2 test).

Results

Three commercially available trap designs were tested in cacao fields at the Cocoa Research and Development Center, Malaysian Cocoa Board, Sabah, Malaysia, using an NRI synthetic sex pheromone formulation. Although very low populations of adult *C. cramerella* moths were present, a total of 70 males were captured during 7 d from 6 to 12 August 2005, confirming that the synthetic pheromone was attractive to male *C. cramerella* in Sabah. Delta traps captured significantly more male *C. cramerella* than did Pherocon V scale traps (Fig. 1; $N = 3$; $F = 5.39$; df = 2,6; $P < 0.05$), indicating that the Delta traps are suitable traps for male *C. cramerella* captures in the field conditions.

Field trapping studies were then conducted in four different locations using Delta traps in the Cocoa Research and Development Center, Sabah, Malaysia. A total of 1,195 male *C. cramerella* were captured during a ≈4-mo trapping period in late 2005 (Table 1).

In two fields at the Cocoa Research and Development Center located in peninsular Malaysia, populations of adult *C. cramerella* moths in one field were very low. A total of 232 male *C. cramerella* were captured during a 3-mo trapping period in late 2005 (Table 1).

Field trapping studies were conducted in two adjacent commercial plots in Teck Guan Estate, Sabah, Malaysia, in which the populations of *C. cramerella* were higher than the cacao fields at the Cocoa Research and Development Center. During a 1-yr trapping period from early 2006 to early 2007, a total of 22,690 male *C. cramerella* were captured in these two plots (Table 1).

Pheromone activity evaluation was also conducted in four different cacao fields in Sulawesi, Indonesia. A total of 2,055 male *C. cramerella* was captured in the traps during 3- to 8-mo trapping periods in 2006–2007 (Table 1).

Populations of *C. cramerella* were monitored in 12 different plots in Rambong Sialang Estate, Sumatra, Indonesia. A total of 7,729 male *C. cramerella* were captured in the traps during a period of 5 wk in 2006. Our data on the number of male *C. cramerella* captured in the traps baited with sex pheromone blend showed that lure attractiveness did not change in the first 4 wk, but it decreased quickly in the fifth week (Fig. 2).

To evaluate lure longevity in the field, the trap capture data obtained from two commercial cacao plots in Teck Guan Estate, Sabah, Malaysia, during a period of 16 mo from 9 November 2005 to 6 March 2007 were analyzed on a monthly basis. During this trial, the population of *C. cramerella* was very high compared with other locations. A total of 27,328 male *C. cramerella* were captured in pheromone-baited traps. In most cases, trap captures reached a peak within 1 mo, after which the *C. cramerella* counts decreased rapidly (Fig. 3).

Lure release rate and longevity of *C. cramerella* synthetic sex pheromone were also studied under controlled conditions in a laboratory fume hood, and remaining pheromone residues were determined using

Table 1. Number of male *C. cramerella* caught in the traps baited with pheromone blend and blank control in Malaysia and Indonesia

Location		Time	Pheromone	Control	χ^2	df	*P*
Malaysia							
Sabah, MCB	Mile 10	23 Aug 05 to 4 Jan 06	100	5	86	1	<0.001
	Madai	13 Sep 05 to 5 Jan 06	249	0	249	1	<0.001
	Kg. Ranggu	23 Aug 05 to 4 Jan 06	277	0	277	1	<0.001
	Kau Sing	23 Aug 05 to 4 Jan 06	564	0	564	1	<0.001
Sabah, Teck Guan Estate	Field 5	9 Feb 06 to 6 Feb 07	13,289	181	2,800	1	<0.001
	Field 47	9 Feb 06 to 6 Feb 07	9,143	77	8,910	1	<0.001
Peninsular, MCB	Hilir Perak	9 Sep to 25 Dec 05	204	6	187	1	<0.001
	Kg. Lekir	9 Sep to 25 Dec 05	21	1	18.2	1	<0.001
Indonesia							
Sulawesi, Effem Foods SE	Wonosari	16 Jan 06 to 10 Sep 06	1,497	0	1,497	1	<0.001
	Yaminas, Noling	13 Dec 06 to 13 Mar 07	261	3	252	1	<0.001
	Pinrang 1	16 Jan 06 to 4 Jun 06	139	7	119	1	<0.001
	Pinrang 2	16 Jan 06 to 4 Jun 06	140	8	118	1	<0.001

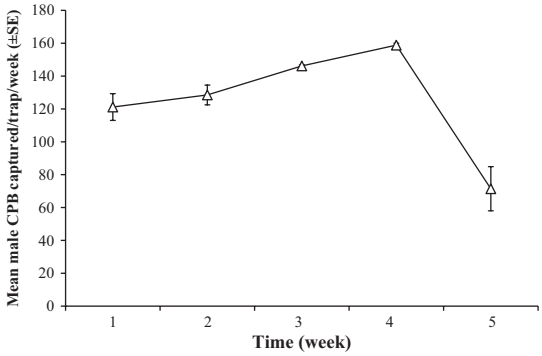

Fig. 2. Mean numbers of male *C. cramerella* (±SE) caught in Delta traps (Pest Control) baited with pheromone blend (Pest Control) at the Rambong Sialang Estate, Sumatra, Indonesia.

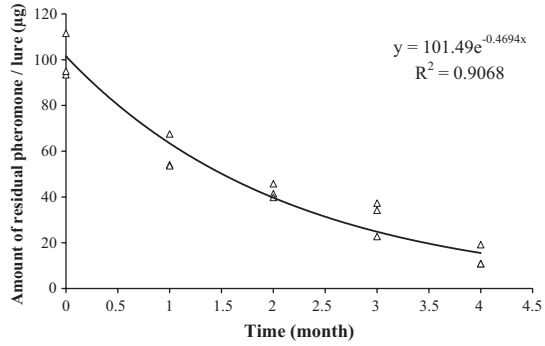

Fig. 4. Residues of *C. cramerella* pheromone detected in polyethylene vials after exposure under fume hood conditions for a period of 4 mo.

GC-MS. We found that volatile active ingredients were desorbed from polyethylene vials following first-order kinetics (Fig. 4; $r^2 = 0.9068$). The decrease of pheromone over time was best described by the following equation: $Y = 101.49e^{-0.4694t}$.

In addition, the effectiveness of different pheromone loadings and formulations was evaluated in two cacao fields in Noling, Sulawesi, Indonesia, using traps produced by Pest Control. A total of 1,419 male *C. cramerella* were captured during a 7-wk trapping period from 11 November 2006 to 4 January 2007. There were no significant differences in trap catches among pheromone blends produced by NRI, United Kingdom, or Pest Control, as well as vials produced in the United Kingdom (Just Plastics) or in India (Mic & Mac Plastic Industries). Male *C. cramerella* catch was not significantly different in traps baited with 100-, 300-, or 1,000-μg doses of *C. cramerella* synthetic sex pheromone blend (Table 2; $\chi^2 = 4.46$; df = 5; $P = 0.49$).

Discussion

Since the identification of the sex pheromone of *C. cramerella* in the 1980s, a wide range of trap designs baited with the synthetic pheromone was evaluated in the cacao fields in Malaysia, and the sandwich trap, also called lobster trap, was determined to be the most suitable trap design for the male *C. cramerella* moth catch (Beevor et al. 1986b, Ho et al. 1987). However, this custom-made trap is not commercially available, and the polybutene glue used for these traps had to be applied in the field. In addition, the stickiness of the glue lasted only up to 4 wk under field conditions (Ho et al. 1987). Therefore, three different trap designs that are commercially available were re-evaluated, so that an effective and suitable trap design could be selected for the rest of our field studies. Because the Delta trap is easier to service, having a removable liner, and because the trap is reusable, it was chosen to be used for the rest of field trials.

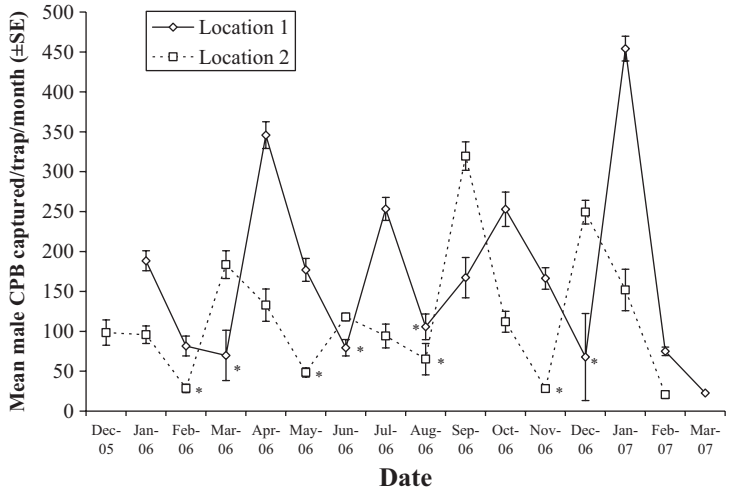

Fig. 3. Mean numbers of male *C. cramerella* (±SE) caught in traps (Trécé) baited with synthetic sex pheromone blend (NRI) at the Teck Guan Estates, Sabah, Malaysia over a period of 16 mo. *Replacements of old pheromone lures with new lures.

Table 2. Mean numbers (log scale) of male *C. cramerella* caught in the traps baited with different pheromone formulations in Noling, Sulawesi, Indonesia

Formulation	No. male *C. cramerella* captured per week (± SE)
PCIL 100 μg/United Kingdom	2.4763 ± 0.2231a
PCIL 300 μg/United Kingdom	2.5619 ± 0.2209a
PCIL 1000 μg/United Kingdom	2.7027 ± 0.2195a
PCIL 100 μg/INa	2.3336 ± 0.2243a
PCIL 100 μg/INb	2.1568 ± 0.2270a
NRI 100/United Kingdom	2.1940 ± 0.2262a

Means within column followed by the same letter are not significantly different at $P < 0.05$.

Our study results clearly showed that the same pheromone blend was not only attractive in both Sabah and peninsular Malaysia but also attractive in different locations in Indonesia (Table 1), confirming that the same pheromone strain of *C. cramerella* existed throughout our study area, in contrast with earlier assumptions. This will be beneficial information for commercialization of the *C. cramerella* pheromone in Asia.

Based on the dose–response data obtained from Sulawesi, Indonesia, it seems that a 100-μg dose reached the optimum loading of sex pheromone blend in the polyethylene vials used for the field attraction, and higher doses neither increased nor decreased the catch of male *C. cramerella* (Table 2).

In Sumatra, Indonesia, it seems that a dose of 100 μg of pheromone/polyethylene can produce maximum attraction for a period of 1 mo (Fig. 2). This result is consistent with the trapping data obtained in Sabah, Malaysia, in that attractiveness of the sex pheromone lure used was ≈1–2 mo in the field (Fig. 3). During the course of experiments in Sabah, Malaysia, the liners were changed when necessary; the traps themselves, however, were never changed, indicating that the Delta traps from Trécé are suitable for use for >16 mo under the conditions of high temperature, humidity, and rainfall in Sabah.

Under laboratory conditions, pheromone components desorbed from polyethylene vials follow first-order kinetics ($Y = 101.49e^{-0.469x}$), and the rate constant (k) is equal to 0.4694 (Fig. 4). Therefore, the half-life time ($t_{1/2} = 0.693/k$) of the lures is equal to ≈1.5 mo (44 d). Theoretically, the half-life time ($t_{1/2}$) of synthetic sex pheromone lures was not dependent on the original loading in the formulations. Therefore, the lures with 100-, 300-, and 1,000-μg loadings will release 50% of pheromone in ≈44 d. According to the trap capture data of male *C. cramerella* in Malaysia (Fig. 3) and Indonesia (Fig. 2), attractiveness of new *C. cramerella* lures at 100-μg loading decreased significantly after 1-mo exposure in the fields. Thus, the minimum threshold of sex pheromone dose after 1-mo exposure in the field is ≈62 μg/lure (Fig. 4). We hypothesize that the lures used in this study over time can be described by the following equation: $62 = Y_o e^{-0.4694 t_{max}}$ (Y_o is the original loading [μg] of pheromone in polyethylene vials); the maximum attraction time (t_{max}) of the lure can be easily estimated. Therefore, with 1,000-μg pheromone loading, $t_{max} = 5.9$ mo. Correspondingly, with 300-μg pheromone loading, $t_{max} = 3.4$ mo. Considering that the overall temperature in the cacao fields is higher than in our laboratory, the actual rate constant (k) could be >0.4694. Thus, the maximum attraction time (t_{max}) in field conditions may be shorter than the calculated values. In other words, lures baited with 1,000 μg pheromone will be expected to last ≈4–5 mo and lures with 300-μg pheromone loading will be expected to last ≈2–3 mo for maximum attraction in field conditions. Further research, including analysis of amounts of residual pheromone components from lures aged in the field with different doses at different conditions and correlations of pheromone amounts with trap catches, is needed to check this hypothesis.

In conclusion, our research results had shown that the presence of a single pheromone strain of *C. cramerella* in a wide geographic region extending throughout the Indo-Malayan archipelago. Further research is needed to clarify if more than one strain of *C. cramerella* exists in Southeast Asia on cocoa and also on other alternate hosts. Thus far, genetic analysis of the *C. cramerella* moths collected from Malaysia, Philippine, Papua New Guinea, and the islands of Indonesia indicates that the *C. cramerella* populations in cacao fields are surprisingly homogeneous, at least with respect to mitochondrial DNA (L. Shapiro, personal communication). Commercial quantities of pheromones available now with better quality control and trap design should enable their large-scale testing and use for managing *C. cramerella* in Asia. Development of cost-effective pest management strategies should also include pheromone-based monitoring, mass trapping, and possibly mating disruption at a later date in addition to regular and complete harvesting, rational use of effective pesticides, biological control, and plastic sleeving.

Acknowledgments

We thank J. Nie of the Chemicals Affecting Insect Behavior Laboratory, USDA–ARS, for assistance with lure preparation, M. Kramer of the Biometrical Consulting Service, USDA–ARS, for assistance with statistical analysis, B. Lumsden of the World Cocoa Foundation for review of this paper, and R. Bateman of the Imperial College for valuable information. We also acknowledge Mars for supporting this project under the cooperative agreement with USDA–ARS, Plant Sciences Institute, Beltsville Agricultural Research Center, Beltsville, MD.

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Received 2 October 2007; accepted 4 March 2008.