Bean Leaf Beetle (Coleoptera: Chrysomelidae) Management for Reduction of Bean Pod Mottle Virus

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J. Econ. Entomol. 97(2): 192–202 (2004)

ABSTRACT Bean pod mottle virus (BPMV) is a management concern for soybean, *Glycine max* (L.), producers in the North Central states because it can cause yield loss and reduce seed quality by induction of seed coat mottling. The main vector of BPMV is the bean leaf beetle, *Cerotoma trifurcata* (Forster). An experiment was conducted in 2000 and 2001 at two locations in northwestern and central Iowa to test three insecticide treatments for suppression of bean leaf beetles, and subsequently, BPMV. Treatments of insecticide applications with lambda-cyhalothrin were 1) a single early-season application (23 g [AI]/ha) (2.5 oz/acre) at the VE–VC soybean developmental stage; 2) two early-season applications, the first the same as treatment 1 and a second at the same rate 9–13 d later; 3) a single early-season application the same as treatment 1, followed by a mid-season application (28 g [AI]/ha (3.2 oz/acre) at approximately R2 (flowering, near 15 July); and 4) an unsprayed control. Application of lambda-cyhalothrin after soybean emergence and again as first-generation bean leaf beetles emerged in northwestern Iowa in 2000 (treatment 3) significantly reduced beetle densities through mid-season, BPMV field incidence by 31.5%, and seed coat mottling by 31.2%, compared with the unsprayed control. Similar effects were measured at the same location when insecticide was applied twice at early season (treatment 2). Yield was 453.7 kg/ha (6.74 bu/acre) greater in treatment 2 and 525.20 kg/ha (7.80 bu/acre) greater in treatment 3 than in the unsprayed control at the northwestern site in 2000. At both locations in 2001 fewer treatment effects were observed, which was likely related to lower beetle populations in that year. Early-season insecticide sprays targeted at overwintered beetles on VC–VE reduced the initial population of vector insects and may have contributed to a lower first-generation population because of reduced overwintered beetle oviposition. In 1 year at one location there was a benefit to an additional mid-season insecticide spray, although effectiveness of spraying at this time could vary based on the magnitude of the vector population.

KEY WORDS *Cerotoma trifurcata*, soybean, *Glycine max*, insect vector, plant disease

IN 1945, THE FIRST RECORD of bean pod mottle virus (BPMV) was identified from *Phaseolus vulgaris* L. from South Carolina (Zaumeyer and Thomas 1948). BPMV is a major virus of soybean, *Glycine max* (L.), that has been widespread in Mississippi (Pitre et al. 1979), North Carolina (Ross and Butler 1985), Kentucky (Ghabrial et al. 1990), and Iowa (Krell et al. 2003a). In Arkansas, BPMV was described as the most important soybean virus (Hopkins and Mueller 1984) and as the most prevalent soybean virus in Louisiana (Horn et al. 1973). BPMV was not reported in the North Central states until 1968 from Iowa (Quiniones and Dunleavy 1971); however, recently it was documented as widespread in several North Central states (Rice et al. 2000, Giesler et al. 2002, Krell et al. 2003a). BPMV is a concern for soybean growers because it can cause yield losses >50% (Hopkins and Mueller 1984) and seed coat mottling (Stace-Smith 1981, Lin and Hill 1983), which can result in financial loss. In 1999, it was estimated that 155,778 metric tons of soybean yield was lost to soybean viruses in Iowa (Wrather et al. 2003).

The main BPMV vector is the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae) (Hopkins and Mueller 1983, Pitre 1989). This beetle is common on soybean in the North Central states, and in central Iowa its populations have increased to the greatest abundance recorded in 14 yr (Krell et al. 2003a). There are two generations of bean leaf beetle in Iowa (Smelser and Pedigo 1991), and each generation takes ≈1 mo to develop from egg to adult (Isely 1930).

BPMV also can occur with soybean mosaic virus (SMV), and the combined effect of both viruses is synergistic (Calvert and Ghabrial 1983; Ross 1963, 1969) causing yield losses >80% (Ross 1963). SMV is aphid-transmitted, but most native aphids only feed occasionally on soybean. Recently, the soybean aphid, *Aphis glycines* Matsumura, an exotic pest that colonizes soybean, has become extremely abundant in

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some North Central states (Rice 2000). The potential for dual infection by these two viruses has been heightened because *A. glycines* can reach extremely high populations on soybean and transmits SMV. Other pathogens that have been associated with BPMV are fungi in the genus *Phomopsis* (Stuckey et al. 1982, Abney and Ploper 1994), which are considered one of the most important causes of seed disease in soybean.

Despite the history of BPMV in the United States and its potential impact, there are no proven management recommendations for reducing BPMV incidence in soybean (Gergerich 1999). The ideal BPMV management tactic would be soybean resistance to the virus. Soybean BPMV resistance has been explored, but no resistance is known in conventional soybean varieties (Skotland 1958, Scott et al. 1974, Schwenk and Nickell 1980). There has been success in generating transgenic soybean resistance to BPMV (Di et al. 1996, Reddy et al. 2001); however, this trait has not been incorporated into commercially available varieties.

One BPMV management option for soybean growers is to reduce the population density of the bean leaf beetle. Before BPMV became a concern in the North Central states, the bean leaf beetle was primarily managed late in the season only when populations exceeded economic thresholds for soybean pod feeding. When large populations of bean leaf beetles occur in the presence of BPMV, early-season vector suppression may be important and management tactics should be timed to correspond to specific events in beetle and soybean phenology.

The bean leaf beetle overwinters as an adult (Isely 1930) primarily in wooded areas (Lam and Pedigo 2000). In early spring it leaves overwintering sites and moves to feed on naturally occurring legumes and alfalfa (Isley 1930, Kogan et al. 1980, Smelser and Pedigo 1991). At least one native legume, *Desmodium canadense* (L.), has been identified as a naturally occurring host of BPMV (Krell et al. 2003a) and bean leaf beetles are known to feed on this species (R.K.K., unpublished data). As soon as soybean seedlings emerge, beetles move to them and begin feeding (Smelser and Pedigo 1991). Typical BPMV seed transmission rates are <1% (Lin and Hill 1983, Krell et al. 2003a) and the virus may be carried by overwintered bean leaf beetles and transmitted the following spring at <2% without further acquisition (Krell et al. 2003a). Therefore, early-season inoculum sources, whether through seedborne infections or from plants inoculated by overwintered beetles, are readily available for subsequent spread of BPMV.

Application of insecticides after soybean emergence to reduce early-season inoculation and spread of BPMV by bean leaf beetles has been suggested (Hopkins and Mueller 1984, Ross 1986, Ghabrial et al. 1990); however, field evaluation of this management tactic has not been reported. The emphasis on early-season management is suggested because bean leaf beetles feed on soybean as soon as cotyledons emerge, and there is a strong positive relationship between plant age at infection and yield reduction (Ross 1969, Walters 1970, Hopkins and Mueller 1984, Ragsdale 1984).

Instead of an early-season application, Hopkins and Mueller (1983) suggested application of insecticides in the middle of the soybean-growing season. Their suggestion was based on their discovery of the greatest increase in BPMV incidence after the peak of the first-generation beetle population and recording the most viruliferous beetles (16%) at mid-season. Calvert and Ghabrial (1983) also reported the greatest increase in secondary spread of BPMV at 76–89 d after planting, which would correspond to a period after first-generation beetle emergence. Both these studies suggest that control of first-generation bean leaf beetles at mid-season may be important for reducing BPMV incidence at a time when soybean is still susceptible to the effects of disease. Although yield reductions were found to be greatest with inoculation at soybean vegetative stages, yield reductions as high as 15.1% were found in soybean inoculated at the R6 plant stage (Hopkins and Mueller 1984).

Based on knowledge of the bean leaf beetle life cycle and virus epidemiology, we tested three insecticide management regimes for effect on bean leaf beetle density and subsequent BPMV incidence. The objective was to develop a management tactic for BPMV that could be readily implemented by growers using existing soybean pest management technology.

Materials and Methods

Field Sites. The experiments were performed in 2000 and 2001 at two Iowa field sites known to have had large bean leaf beetle populations and apparent symptoms of BPMV. The central site was located in Ames at the Iowa State University Ross Farm in 2000 and at the Accola Farm in 2001. The northwestern site was located on the Linn Farm in Correctionville in 2000 and on Iowa State University's Allee Research Farm in Newell in 2001. A food-grade soybean (Vinton 81) was planted at the central site in both years. At the northwestern site, a commodity soybean variety (MRK 9823) was planted in 2000. In 2001, the variety at the northwestern site was the same as in 2000, except that it contained a trait for resistance to soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (MRK 9923CTA) because SCN was known to occur in the field. The field in the central site was planted on 15 May 2000 and on 11 June 2001, and the field in the northwestern site was planted on 3 May 2000 and 15 May 2001. Planting dates in 2001 were delayed because of heavy early-season rain. All fields were planted in 76.2-cm (30-in.) row spacings. Weed control was implemented, as needed, following standard agronomic practices.

Treatments. Each treatment plot was at least 152.4 by 36.8 m (48 rows in width), and plots were arranged in a randomized complete block design with four replicates. Because beetles are known to make trivial flights of 11 m (Krell et al. 2003b), each plot was at least 0.56 ha (1.38 acre) to reduce the possibility that beetles might move between sample areas in each plot. Only the middle 20 rows of each plot were sampled.

The insecticide regimes tested used lambda-cyhalothrin (Warrior) (Syngenta, Wilmington, DE) because it provides relatively long suppression (21 d) of adult bean leaf beetle density (Hammond 1996). Treatments were 1) a single early-season application of lambda-cyhalothrin (23 g [AI]/ha) (2.5 oz/acre) at the VE–VC (Fehr et al. 1971) soybean developmental stage; 2) an application of lambda-cyhalothrin (23 g [AI]/ha) (2.5 oz/acre) at the VE–VC stage and a second 9–13 d later; 3) an early-season lambda-cyhalothrin application at the same rate and time as the single early-season application (treatment 1), followed by a mid-season application at the maximum rate (28 g [AI]/ha) (3.2 oz/acre) at approximately R2 (near 15 July) (Fehr et al. 1971); and 4) an untreated control. The mid-season insecticide was applied when the first teneral bean leaf beetles were detected in the field, indicating the emergence of the first generation. Early-season applications were designed to prevent initial BPMV transmission, and the mid-season application was designed to reduce virus spread by first-generation beetles.

Insecticide Efficacy. To evaluate the efficacy of the insecticide treatments, bean leaf beetles were sampled weekly. At VE–V4, in situ counts were made in each plot by examining every plant in 5 m of row. After V4, sweep-net samples (50 sweeps per sample) within a single row were taken down the row using a sweep net (38 cm in diameter). A different row was sampled each week. Each sample was placed in a plastic bag and frozen (−20°C) until beetles could be counted.

Immunological Assay. Immunological assays were performed to test soybean tissue and bean leaf beetles for presence of BPMV coat protein, indicating BPMV infection. For all immunological assays, a biotin-avidin double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used. The ELISA was similar to that described previously (Diaco et al. 1985), except that wells of Immulon 1B polystyrene microtiter plates (Dynex Technologies Inc., Chantilly, VA) were coated with anti-BPMV polyclonal antibody prepared to the I-JH1 BPMV isolate (Gu et al., 2002) (1.0 μg/ml) and biotinylated polyclonal anti-BPMV was used at 0.5 μg/ml. Alkaline-phosphatase conjugated extravidin (1:40,000) (Sigma-Aldrich, St. Louis, MO) was followed by *p*-nitrophenyl phosphate (1 mg/ml). Samples were positive if the absorbance value of duplicate wells was greater than twice the standard deviation plus the mean of the negative control (sap from healthy soybean leaves of plants grown in the greenhouse).

Additionally, ELISA to test for SMV antigen content was performed on a 100-seed sample from each treatment as described by Steinlage et al. (2002). Tests for SMV were performed to discern whether interactions between the two viruses might be a factor affecting results from field plots.

BPMV Incidence. Leaf samples were taken at VC, R3–4, R6, and R7 (Fehr et al. 1971) to determine BPMV incidence. Samples were taken at VC to determine whether BPMV was present early in soybean development. For VC samples, single unifoliolate leaves were removed from different plants at equal intervals within a 5-m length of row. At the central site, five unifoliolate leaves were collected per treatment in both years, and at the northwestern site, two unifoliolate leaves in 2000, and five unifoliolate leaves in 2001, were collected from separate plants in each treatment. In 2000, unifoliolate samples were taken 2 d after the insecticide application at the central site and 10 d after the application at the northwestern site. In 2000, there were no significant differences in BPMV incidence detected from unifoliolate samples after the first insecticide application at either site; therefore, data were combined across all treatments to determine early-season whole-field BPMV incidence. In 2001, unifoliolate samples were taken before the first insecticide application and were combined across all treatments to determine whole-field BPMV incidence. At other plant stages, 10 trifoliolate leaves were blindly chosen from each replication of each treatment in a single middle row at five-step intervals until 10 leaves were collected. Sap was extracted individually from each leaf sample in phosphate-buffered saline (PBS) (0.05 M sodium phosphate, pH 7.0, containing 0.15 M NaCl) by using a sap extractor (Ravenel Specialties Corp., Seneca, SC). Samples were frozen (−20°C) until testing by ELISA.

On the same sample dates when trifoliolates were collected for BPMV tests, bean leaf beetles were saved from sweep-net samples to evaluate the percentage virus-carrying beetles by treatment. If there were $<$20 beetles per sample, then all beetles were saved. In samples with $>$20 beetles, only 20 beetles were saved for BPMV testing. Beetles were frozen (−20°C) and macerated individually in 1.5-ml microcentrifuge tubes with 1 ml of PBS. Macerated samples were frozen until analysis with ELISA.

Yield and Seed Quality. Seed was harvested from the middle 16 rows of each treatment to determine yield and seed quality. All yield estimates were standardized to 13% moisture content before analysis. A sample ($\approx$3,000 cm^3) was randomly collected from each treatment as the seed was harvested for agronomic and seed quality assessment.

Two evaluations of seed coat mottling were made. A 1-kg seed sample from each treatment was sent to a seed quality analysis facility (Eastern Iowa Grain Inspection and Weighing Services, Inc, Davenport, IA) for analysis of seed coat mottling and seed damage. In the grain quality assay, any single seed with $>$50% seed discoloration was counted as mottled. Any other seed shape or color abnormalities were incorporated into an estimate of damaged seed. As a second measure of seed coat mottling, three 100-seed samples from each field were evaluated, and any seed showing $>$0% seed coat discoloration was counted as mottled.

As another measure of seed quality, three additional 100-seed samples from each treatment were planted in trays in a greenhouse and rated for percentage of emergence after 10 d. In 2000, the treatments showing the lowest and highest emergence were sent to the

Table 1. Mean bean leaf beetle counts (± SE) at central Iowa field in 2000 and 2001

Sample date	One early[a]	Two early[b]	One early, one mid[c]	Unsprayed control
	Year 2000			
5/30[d]	2.75 ± 0.48a	6.75 ± 1.75a	5.50 ± 1.94a	5.00 ± 1.22a
6/08	0.25 ± 0.25a	0.00 ± 0.00a	1.00 ± 0.71a	1.25 ± 0.75a
6/14	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	1.25 ± 0.48a
6/26	0.00 ± 0.00b	0.00 ± 0.00b	0.50 ± 0.29ab	1.75 ± 0.75a
7/06[e]	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	1.25 ± 0.48a
7/12	16.75 ± 2.78a	18.25 ± 2.43a	16.25 ± 0.48a	19.00 ± 4.83a
7/19	52.25 ± 14.33a	29.75 ± 9.78ab	2.25 ± 1.31b	61.00 ± 18.46a
7/27	34.75 ± 10.82a	47.25 ± 18.85a	22.75 ± 6.74a	51.50 ± 11.77a
8/02	38.00 ± 16.98a	61.25 ± 40.58a	57.25 ± 20.09a	91.25 ± 40.61a
8/09	66.50 ± 17.79a	48.50 ± 14.38a	64.50 ± 24.18a	69.25 ± 18.19a
8/17	34.00 ± 13.21a	46.00 ± 12.48a	53.00 ± 14.75a	87.75 ± 35.82a
8/24	142.50 ± 51.90a	135.00 ± 42.63a	141.25 ± 40.90a	127.00 ± 33.79a
8/30	151.50 ± 38.72a	214.00 ± 91.10a	74.25 ± 26.17a	144.00 ± 39.94a
9/06	128.50 ± 31.92a	124.25 ± 60.75a	59.75 ± 14.52a	129.75 ± 46.12a
9/15	98.25 ± 47.27a	52.25 ± 17.24a	30.50 ± 14.56a	138.50 ± 75.25a
	Year 2001			
6/18[d]	0.00 ± 0.00a	0.00 ± 0.00a	0.25 ± 0.25a	0.25 ± 0.25a
6/25	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.25 ± 0.25a
7/02[e]	0.00 ± 0.00a	0.25 ± 0.25a	0.00 ± 0.00a	0.25 ± 0.25a
7/09	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.25 ± 0.25a
7/16	0.25 ± 0.25a	0.25 ± 0.25a	0.25 ± 0.25a	0.75 ± 0.48a
7/25	32.00 ± 5.61a	39.00 ± 9.49a	13.50 ± 3.38b	34.25 ± 11.67a
7/30	7.00 ± 3.32a	7.50 ± 3.48a	7.00 ± 1.58a	15.50 ± 7.73a
8/08	6.50 ± 3.23a	4.75 ± 2.50a	13.50 ± 7.37a	8.50 ± 2.53a
8/13	10.75 ± 3.71a	7.75 ± 1.70a	11.74 ± 3.20a	9.00 ± 3.24a
8/20	10.25 ± 3.54a	9.75 ± 2.25a	9.00 ± 2.12a	11.50 ± 5.07a
8/27	175.75 ± 72.47a	148.50 ± 60.13a	30.00 ± 8.78a	183.50 ± 66.04a
9/10	106.75 ± 39.55a	204.75 ± 83.73a	178.50 ± 107.49a	112.50 ± 44.30a
9/17	144.25 ± 53.10a	97.25 ± 15.67a	105.50 ± 26.26a	86.00 ± 34.91a

Means followed by the same letter within each date are not significantly different ($P > 0.05$) (ANOVA).
[a] Insecticide applied on 6 June 2000 and 20 June 2001.
[b] Insecticide applied as in [a] and on 19 June 2000 and 29 June 2001.
[c] Insecticide applied as in [a] and on 14 July 2000 and 17 July 2001.
[d] Mean bean leaf beetle density determined from in situ counts of 5-m soybean row from this date through 26 June in 2000 and 25 June in 2001.
[e] Mean bean leaf beetle density determined from individual 50-sweep samples from this date through last sample date.

Iowa State University Seed Testing Laboratory to test for presence of *Phomopsis* spp.

Data Analyses. Data were analyzed separately by location and year and significant differences were designated at $P < 0.05$. Treatment means for bean leaf beetle density, yield, percentage of BPMV field incidence, percentage of seed coat mottling, percentage of seed damage, and percentage of emergence were compared using analysis of variance (ANOVA). If differences were found, mean separations were performed by comparing the least significant difference (LSD) between treatment means.

Disease progress curves were created from BPMV field incidence data obtained from trifoliolates collected at stages R3–R4, R6, and R7 to examine the change in disease incidence over time (dy/dt). Values for percentage of disease incidence from treatment replicates were used to determine the best model for describing disease progress for each treatment. Five models, including Gompertz, exponential, logistic, monomolecular, and linear were compared using the EPIMODEL (Nutter and Parker 1996) program for best fit to the data. Appropriate data transformations were made for each model to linearize the data. The F statistic, coefficient of determination (R^2), and root mean square error were compared to determine the most appropriate model. Once the best model was identified for disease progress curves, slopes of disease progress by location and treatment were compared using analysis of covariance (SAS Institute 1999).

Results and Discussion

Insecticide Efficacy. There were no significant differences between bean leaf beetle densities taken before insecticide treatments were applied in both years at the central site and in 2001 at the northwestern site (Tables 1 and 2).

Treatment 1: One Early Application. Eight, 20, and 30 d after the single early-season insecticide application, bean leaf beetle densities were significantly lower than in the unsprayed control at the central site in 2000 (Table 1; 14 June: $F = 6.82$; df = 3, 9; $P = 0.0108$; 26 June: $F = 4.05$; df = 3, 9; $P = 0.0446$; 6 July: $F = 6.82$; df = 3, 9; $P = 0.0108$). In 2001 there were no significant differences between beetle densities in the single early insecticide application and the unsprayed control. The planting date in 2001 was delayed because of early-season rain. The delay in planting and the cool, wet early-season weather may have contributed to overall lower beetle densities in this field.

Table 2. Mean bean leaf beetle counts (± SE) at northwestern Iowa field in 2000 and 2001

Sample date	Year 2000			
	One early[a]	Two early[b]	One early, one mid[c]	Unsprayed control
5/24[d]	2.00 ± 0.41b	0.50 ± 0.29b	1.50 ± 0.65b	3.75 ± 0.85a
5/29	2.25 ± 0.75b	0.75 ± 0.48b	1.50 ± 0.96b	7.00 ± 1.35a
6/07	1.00 ± 0.71a	0.25 ± 0.25a	1.00 ± 1.00a	2.00 ± 0.41a
6/14	0.25 ± 0.25a	0.50 ± 0.29a	0.50 ± 0.29a	1.75 ± 0.75a
6/21[e]	3.50 ± 2.22a	0.25 ± 0.25a	3.25 ± 1.49a	3.50 ± 2.02a
6/28	2.25 ± 0.63a	0.25 ± 0.25a	0.50 ± 0.50a	1.50 ± 0.65a
7/04	0.25 ± 0.25a	0.00 ± 0.00a	0.25 ± 0.25a	0.00 ± 0.00a
7/12	3.25 ± 1.49b	2.25 ± 0.75b	5.75 ± 2.43b	28.75 ± 10.18a
7/19	3.00 ± 1.47b	5.50 ± 0.65b	0.00 ± 0.00b	14.75 ± 3.77a
7/26	39.75 ± 13.37ab	18.75 ± 4.03bc	0.00 ± 0.00c	70.25 ± 16.61a
8/02	32.50 ± 5.69a	9.25 ± 1.89b	1.75 ± 1.44b	36.75 ± 7.43a
8/09	106.25 ± 43.91a	13.00 ± 2.94a	26.50 ± 2.79a	73.25 ± 15.35a
8/16	25.00 ± 6.31a	6.50 ± 2.60a	23.75 ± 1.65a	22.25 ± 6.92a
8/23	111.50 ± 35.57a	35.25 ± 9.57a	73.50 ± 13.40a	59.75 ± 12.87a
8/30	13.00 ± 6.79a	8.25 ± 3.74a	21.00 ± 8.59a	16.75 ± 7.49a
9/06	69.50 ± 13.79a	41.74 ± 10.91a	34.00 ± 13.32a	60.50 ± 9.32a
	Year 2001			
5/30[d]	0.00 ± 0.00a	0.00 ± 0.00a	0.33 ± 0.21a	0.00 ± 0.00a
6/06	0.75 ± 0.21a	1.00 ± 0.41a	0.75 ± 0.48a	0.75 ± 0.48a
6/13	0.25 ± 0.25b	0.25 ± 0.25b	0.50 ± 0.50b	4.25 ± 1.25a
6/22	0.25 ± 0.25a	0.00 ± 0.00a	0.00 ± 0.00a	1.25 ± 0.95a
6/26[e]	0.00 ± 0.00b	0.00 ± 0.00b	0.50 ± 0.29b	3.25 ± 1.31a
7/05	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.50 ± 0.29a
7/10	0.50 ± 0.50a	0.50 ± 0.50a	1.00 ± 0.71a	2.00 ± 1.10a
7/17	7.00 ± 5.70a	2.50 ± 1.19a	1.75 ± 0.85a	7.00 ± 3.16a
7/26	31.74 ± 10.98a	24.75 ± 7.26ab	16.00 ± 7.56b	20.25 ± 4.31b
8/01	5.25 ± 2.14a	2.75 ± 1.38a	7.00 ± 2.61a	4.00 ± 1.96a
8/06	4.00 ± 1.47a	7.25 ± 0.95a	7.75 ± 1.11a	13.5 ± 7.98a
8/14	4.00 ± 1.29a	5.25 ± 1.89a	4.00 ± 0.71a	3.25 ± 1.31a
8/23	10.75 ± 5.22a	11.50 ± 3.97a	7.25 ± 1.70a	9.75 ± 3.90a
8/29	50.50 ± 23.82a	41.25 ± 18.63a	16.25 ± 4.87a	50.50 ± 23.82a
9/12	361.50 ± 179.50a	387.80 ± 221.18a	137.80 ± 69.70a	211.80 ± 92.03a
9/19	384.00 ± 203.84a	196.30 ± 92.82a	186.50 ± 60.40a	358.30 ± 159.55a

Means followed by the same letter within each date are not significantly different ($P > 0.05$) (ANOVA).

[a] Insecticide applied on 14 May 2000 and 8 June 2001.

[b] Insecticide applied as in [a] and on 23 May 2000 and 19 June 2001.

[c] Insecticide applied as in [a] and on 15 July 2000 and 17 July 2001.

[d] Mean bean leaf beetle density determined from in situ counts of 5-m soybean row from this date through 14 June in 2000 and 22 June in 2001.

[e] Mean bean leaf beetle density determined from individual 50-sweep samples from this date through last sample date.

At the northwestern site in 2000 beetle densities were significantly lower in the single early treatment 10 and 15 d after the insecticide application (Table 2; 24 May: $F = 6.96$; df = 3, 9; $P = 0.0101$; 29 May: $F = 8.47$; df = 3, 9; $P = 0.0055$) and at 59 and 66 d post-application (Table 2: 12 July; $F = 6.75$; df = 3, 9; $P = 0.0111$; 19 July; $F = 9.54$; df = 3, 9; $P = 0.0037$). The significantly lower beetle density at 59 and 66 d post-application was likely because oviposition by the overwintered bean leaf beetles in the treatment area was reduced in early season. Consequently, beetle populations were low in July when the first-generation beetles emerged. In 2001 at the northwestern site, beetle densities were significantly lower 5 d after the single early-season insecticide spray (Table 2; 13 June: $F = 7.08$; df = 3, 9; $P = 0.0096$) and again 18 d post-application (Table 2: 26 June: $F = 5.63$; df = 3, 9; $P = 0.0189$).

Treatment 2: Two Early Applications. In the treatment receiving two early-season insecticide sprays at the central site in 2000, beetle densities were significantly lower than the unsprayed control 8 d after the first insecticide spray (Table 1; 14 June: $F = 6.82$; df = 3, 9; $P = 0.0108$) and seven and 17 d after the second insecticide spray (Table 1: 26 June: $F = 4.05$; df = 3, 9; $P = 0.0446$; 6 July: $F = 6.82$; df = 3, 9; $P = 0.0108$). At the central site in 2001, there were no significant differences in beetle densities after either insecticide application. This was likely related to later field planting and overall lower beetle densities.

In the twice early-season insecticide treatment at the northwestern site in 2000, beetle densities were significantly lower than the unsprayed control at 10 d after the first insecticide spray (Table 2; 24 May: $F = 6.96$; df = 3, 9; $P = 0.0101$) and 6 d after the second insecticide application (Table 2; 29 May: $F = 8.47$; df = 3, 9; $P = 0.0055$). The beetle density also was significantly lower than the control at 50, 57, 64, and 71 d after the second early-season treatment (Table 2; 12 July: $F = 6.75$; df = 3, 9; $P = 0.0111$; 19 July: $F = 9.54$; df = 3, 9; $P = 0.0037$; 26 July: $F = 7.93$; df = 3, 9; $P = 0.0068$; 2 August: $F = 17.80$; df = 3, 9; $P = 0.0004$). Again, lower beetle densities at this time in the season were likely due to the suppression of oviposition by

Table 3. Mean percentage (± SE) of BPMV incidence, yield, percentage mottled seed, percentage damaged seed, and percentage seed emergence by treatment at two locations in two years

Location and treatment	% BPMV incidence at R6	Yield (kg/ha)	% seed >50% mottled	% seed >0% mottled	% damaged seed	% seed emergence
Central 2000						
one early	67.5 ± 17.0a	1751.8 ± 282.1a	5.2 ± 0.5a	38.0 ± 1.5ab	2.6 ± 0.4a	66.8 ± 3.3a
two early	72.5 ± 10.3a	1663.5 ± 224.5a	5.6 ± 0.9a	40.8 ± 2.8a	1.9 ± 0.2a	61.1 ± 2.8a
one early, one mid	70.0 ± 17.8a	1875.1 ± 344.8a	4.5 ± 0.6a	32.2 ± 2.1b	1.7 ± 0.5a	66.5 ± 2.5a
unsprayed control	92.5 ± 4.8a	1696.7 ± 313.5a	6.5 ± 0.5a	44.2 ± 1.8a	2.9 ± 0.5a	59.8 ± 4.6a
Central 2001						
one early	27.5 ± 4.8a	1209.3 ± 432.9a	3.3 ± 0.6a	35.5 ± 2.2a	6.4 ± 1.4a	84.8 ± 4.1a
two early	45.0 ± 9.6a	1561.7 ± 310.3a	3.4 ± 0.8a	30.3 ± 4.1a	4.7 ± 1.3a	86.5 ± 2.0a
one early, one mid	37.5 ± 8.5a	1401.9 ± 215.1a	1.9 ± 0.5a	22.0 ± 1.9a	2.7 ± 0.4a	86.6 ± 2.5a
unsprayed control	55.0 ± 14.4a	1410.2 ± 150.4a	3.5 ± 0.8a	30.2 ± 2.8a	5.1 ± 1.4a	81.3 ± 1.3a
Northwest 2000						
one early	77.5 ± 8.5a	3146.8 ± 152.9ab	0.2 ± 0.1b	12.5 ± 1.4b	1.9 ± 0.3a	89.4 ± 2.2ab
two early	46.0 ± 9.5bc	3351.4 ± 157.6a	0.3 ± 0.2b	6.2 ± 0.9c	0.6 ± 0.1b	88.8 ± 1.4ab
one early, one mid	38.5 ± 12.6c	3422.9 ± 171.0a	0.3 ± 0.1b	5.3 ± 0.6c	0.5 ± 0.1b	92.3 ± 0.3a
unsprayed control	70.0 ± 4.1ab	2897.7 ± 122.8b	1.9 ± 0.4a	36.3 ± 2.0a	1.6 ± 0.1a	85.5 ± 1.6b
Northwest 2001						
one early	60.0 ± 16.8a	3143.6 ± 220.0a	0.6 ± 0.2a	11.7 ± 1.1ab	0.4 ± 0.1a	89.1 ± 1.6a
two early	50.0 ± 8.2a	3258.1 ± 231.9a	0.4 ± 0.1a	9.2 ± 0.8bc	0.2 ± 0.04a	91.4 ± 1.2a
one early, one mid	40.0 ± 7.1a	3448.8 ± 200.3a	0.9 ± 0.2a	7.7 ± 1.1c	0.3 ± 0.1a	91.0 ± 1.4a
unsprayed control	60.0 ± 12.2a	3092.9 ± 348.4a	0.9 ± 0.3a	14.8 ± 1.6a	0.3 ± 0.1a	92.0 ± 1.1a

Treatment means followed by the same letter within each location and year are not significantly different ($P > 0.05$) (ANOVA).

the overwintering generation because of the timing of early-season insecticide treatments, which reduced the first-generation beetles that were starting to emerge. In the 2001 twice early-insecticide treatment at the northwestern site, beetle density was significantly lower than the unsprayed control at 5 d after the single early-season insecticide spray (Table 2; 13 June: $F = 7.08$; df = 3, 9; $P = 0.0096$) and at 7 d after the second insecticide application (Table 2; 26 June: $F = 5.63$; df = 3, 9; $P = 0.0189$). There were no differences in beetle densities as the first generation beetles emerged in July. It is possible that this effect was not present because beetle populations were lower.

Treatment 3: One Early-, One Mid-Season Application. In 2000, at the central site, in the one-early, one mid-season insecticide spray, the beetle density was significantly lower than the unsprayed control at eight and 30 d after the early-season spray (Table 1; 14 June: $F = 6.82$; df = 3, 9; $P = 0.0108$; 6 July: $F = 6.82$; df = 3, 9; $P = 0.0108$) and at 5 d after the mid-season spray (Table 1; 19 July: $F = 6.95$; df = 3, 9; $P = 0.0102$). In 2001, there were no significant differences in beetle density between treatments after the early-season insecticide spray. The beetle density was significantly lower at 8 d after the mid-season insecticide treatment (Table 1; 25 July: $F = 6.42$; df = 3, 9; $P = 0.0129$). It is likely that differences were not observed between treatments until this date because overall beetle densities had been extremely low; however, this sample date corresponded with the emergence of first-generation beetles and a difference on this date may have been due to a residual repellant effect of the insecticide sprayed 35 d earlier.

At the northwestern site in 2000, beetle densities were significantly lower 10, 15, and 59 d after the early-season insecticide application (Table 2; 24 May: $F = 6.96$; df = 3, 9; $P = 0.0101$; 29 May: $F = 8.47$; df = 3, 9; $P = 0.0055$; 12 July: $F = 6.75$; df = 3, 9; $P = 0.0111$). Beetle densities were significantly lower than the unsprayed control 4, 11, and 18 d after the mid-season insecticide spray (Table 2; 19 July: $F = 9.54$; df = 3, 9; $P = 0.0037$; 26 July: $F = 7.93$; df = 3, 9; $P = 0.0068$; 2 August: $F = 17.80$; df = 3, 9; $P = 0.0004$). In 2001, beetle densities were significantly lower than the unsprayed control 5 d after the single early-season insecticide spray (Table 2: 13 June; $F = 7.08$; df = 3, 9; $P = 0.0096$) and again 18 d postapplication (Table 2; 26 June: $F = 5.63$; df = 3, 9; $P = 0.0189$). There were no significant differences in beetle densities between treatments after the mid-season application. Overall, beetle densities remained low at this location until the second generation began to emerge in late August, which likely accounted for the lack of treatment differences at mid-season.

Treatment Summary. At the central site in 2000, the single early- and twice early-season insecticide treatments provided similar reduction in beetle densities. The one early-, one mid-season treatment was similar, except that it provided one additional date of beetle reduction at mid-season. At the northwestern site in 2000, the three treatments provided similar reduction in beetle density, except the twice early- and one early-, one mid-season treatments provided two additional weeks of beetle suppression at mid-season. Also, the beetle density in the one early-, one mid-season treatment was lower than that in the twice early-season treatment and was significantly lower on 26 July (Table 2; $F = 7.93$; df = 3, 9; $P = 0.0068$).

In 2001, overall densities of overwintering and first generation beetles were lower at both locations, which was likely because planting date was delayed because of cold, wet weather. Delayed planting has been correlated with lower early-season beetle densities (Pedigo and Zeiss 1996), which may have less-

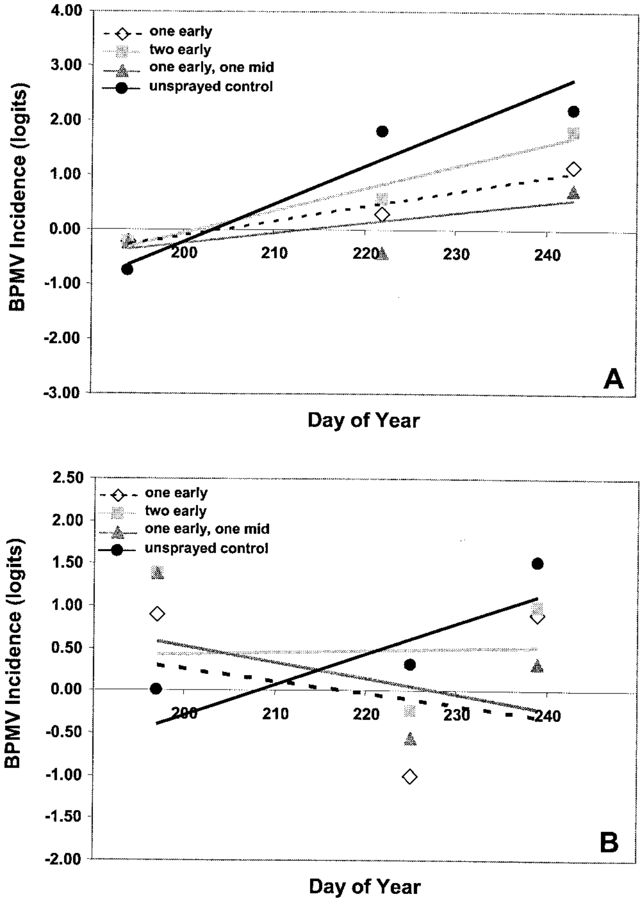

Fig. 1. Disease progress curves derived from logit transformation of disease incidence by treatment at central Iowa site. Incidence data reported are means and model parameters estimated from replicate data where $0 < y < 1$. (A) 2000. (B) 2001.

ened the impact of treatments targeting the feeding and oviposition habits of overwintered populations and, because beetle densities were already low, the impact of feeding by the first-generation beetles.

In general, the early-season treatments conferred similar beetle suppression in both years at both locations, but the mid-season treatment provided the most significant reduction in beetle densities the latest in the season.

BPMV Incidence. In VC unifoliolate samples from the central site, 57.5 ($n = 80$) and 88.8% ($n = 80$) were positive for BPMV in 2000 and 2001, respectively. In the VC samples from the northwestern site, 25.0 ($n = 32$) and 54.3% ($n = 94$) were positive in 2000 and 2001, respectively. Overall, a higher than expected BPMV incidence was detected early in the season, supporting the hypothesis that early-season beetle suppression could be important for BPMV reduction because sources of inoculum are abundant. These results also suggest that early-season insecticide sprays to reduce BPMV should be applied as near to soybean emergence as possible to limit early-season transmission and spread.

At developmental stage R6, the percentage of BPMV-infected plants was significantly lower in the treatment receiving one early- and one mid-season insecticide application at the northwestern site in 2000 (Table 3; $F = 5.99$; df = 3, 9; $P = 0.0158$). The percentage of BPMV incidence in the one early-, one mid-season application treatment at the northwestern site was 38.5%, indicating that BPMV incidence did not exceed the economic threshold (40%) reported for BPMV (Horn et al. 1973).

In general, the percentage of bean leaf beetles in which BPMV antigen was detected was not a useful measure of virus incidence in the experimental plots. Beetle samples collected at the two sites at R3–R4, R6, and R7 plant stages and analyzed by ELISA for virus antigen did not show a consistently significant pattern across all treatments and sites (data not shown). This may reflect movement of beetle vectors among the plots, despite the relatively large plot size.

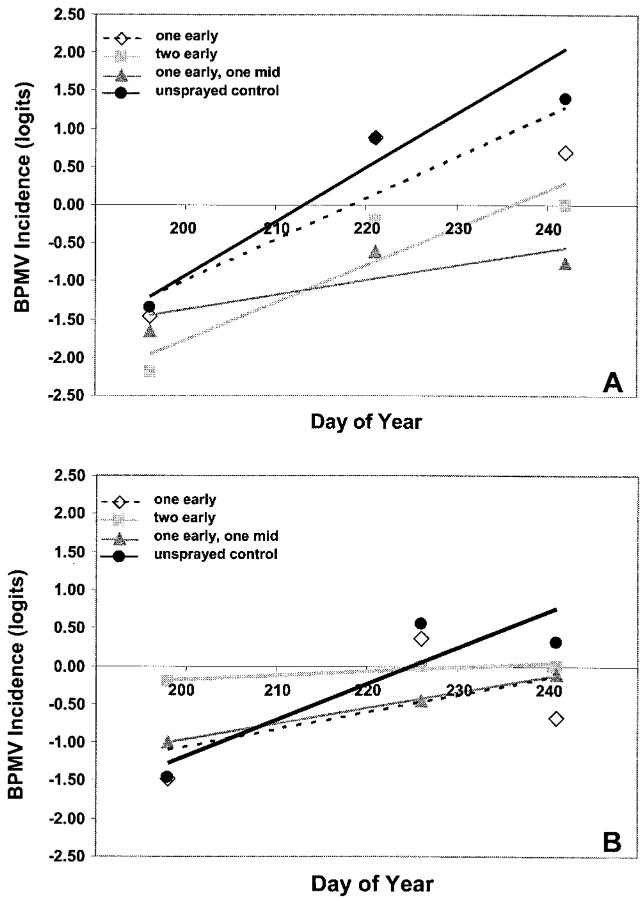

Fig. 2. Disease progress curves derived from logit transformation of disease incidence by treatment at northwestern Iowa site. Incidence data reported are means and model parameters estimated from replicate data where $0 < y < 1$. (A) 2000. (B) 2001.

A single model did not consistently describe all data for disease progress curves; however, the logistic model fit most of the data well and is a standard model that has fewer assumptions and more direct interpretation than other models (Epstein et al. 1997). Therefore, the logistic model was chosen as the best description of the data. Data were transformed to logits to linearize the data. BPMV incidence increased over time at both locations; however, the rates of increase differed, although not significantly, by treatment (Figs. 1 and 2; Table 4). The rate of increase in disease during the season was greatest in the unsprayed control and typically lowest in the treatment receiving one early- and one mid-season application (Figs. 1 and 2); however, the slopes were not significantly different than any of the treatments (Table 4). Future studies of BPMV progress over time should take samples on more sample dates, especially early in the season, to derive a better prediction of disease progress.

Soybean mosaic virus was not detected from any of the seed tested, indicating interactions between SMV and BPMV were not a factor in evaluating field results.

Yield and Seed Quality. At the northwestern location in 2000, yield was significantly higher in the treatment receiving two early-season insecticide applications and in the one early- and one mid-season application (Table 3; $F = 5.02$; df = 3, 9; $P = 0.0257$). The yield protection conferred at the northwestern site in the twice early- and one early-, one mid-season treatments was enough to pay for the cost of the insecticide treatment. Applying twice early would have cost approximately \$32.00/ha (\$12.80/acre) and applying once early and once mid-season would have cost approximately \$35.00/ha (\$14.00/acre). The average price of soybean, during the study years was \$0.17/kg (\$4.50/bu). Therefore, the gain threshold (Stone and Pedigo 1972) for the twice early treatment was 188.23 kg/ha (2.84 bu/acre) and for the one early-, one mid-season treatment was 205.88 kg/ha (3.11 bu/acre). The protection conferred by the treatments exceeded those minimums because yield was 453.7 kg/ha (6.74 bu/acre) greater in the twice early treatment and 525.2 kg/ha (7.80 bu/acre) greater in the one early-, one mid-season treatment than in the control. Of the two treatments conferring yield protection, the one early-,

Table 4. Logistic model parameters and statistics describing the progress of BPMV at two locations in Iowa in 2000 and 2001. Slopes were not significantly different ($P < 0.05$) between treatments by year and location

Location and treatment	Intercept	Slope	R^2	SEE_y[a]	P
Central 2000					
one early	−5.47	0.027	0.30	0.01	0.1017
two early	−8.16	0.041	0.73	0.01	0.0009
one early, one mid	−4.03	0.019	0.18	0.01	0.2179
unsprayed control	−14.18	0.070	0.86	0.01	0.0024
Central 2001					
one early	3.11	−0.014	0.04	0.03	0.6225
two early	0.03	0.002	0.01	0.04	0.9561
one early, one mid	4.28	−0.019	0.07	0.03	0.5321
unsprayed control	−7.55	0.036	0.16	0.04	0.3796
Northwest 2000					
one early	−11.82	0.054	0.64	0.02	0.0094
two early	−11.51	0.049	0.56	0.01	0.0053
one early, one mid	−5.15	0.019	0.12	0.02	0.2908
unsprayed control	−15.08	0.071	0.66	0.02	0.0081
Northwest 2001					
one early	−5.45	0.022	0.07	0.03	0.4223
two early	−0.84	0.004	0.01	0.01	0.7590
one early, one mid	−5.06	0.020	0.23	0.01	0.1154
unsprayed control	−10.64	0.047	0.44	0.02	0.0268

[a] Standard error for the estimate of y.

one mid-season treatment exceeded the gain threshold by more kilograms per hectare than the twice early treatment, suggesting it was the best treatment for protecting yield.

The percentage of seed with >50% seed coat mottling was significantly lower than the control in all insecticide treatments at the northwestern site in 2000 (Table 3; $F = 13.65$; df = 3, 9; $P = 0.0011$). In 2000, the percentage of seed with seed coat mottling >0% was significantly lower than the control at the central site in the one early-, one mid-season treatment (Table 3; $F = 4.53$; df = 3, 9; $P = 0.0337$), and was significantly lower in all insecticide treated plots at the northwestern site (Table 3; $F = 91.77$; df = 3, 9; $P < 0.0001$). In 2001, the percentage of seed with >0% seed coat mottling was significantly lower than the control in the twice early- and one early-, and one mid-season treatments (Table 3; $F = 6.36$; df = 3, 9; $P = 0.0133$). Overall, the twice early- and one early-, one mid-season treatments had the most consistent effect on reducing the incidence of mottled seed.

The percentage of damaged seed was low. There were no significant differences in the percentage of damaged seed at the central location, but it was significantly lower in the twice early-, and one early- followed by one mid-season application at the northwestern site in 2000 (Table 3; $F = 14.64$; df = 3, 9; $P = 0.0008$). Because overall seed quality was higher (less mottling and less damage), the actual value of the soybean in the twice early- and one early, one mid-season applications may have been greater (e.g., if sold for seed). One-hundred seed-weights were lowest in both years in the control at both locations, but there were no significant differences at either location (data not shown).

The percentage of emerged seed was significantly higher than other treatments only in seed from the one early-, one mid-season treatment at the northwestern site in 2000 (Table 3; $F = 4.99$; df = 3, 9; $P = 0.0263$). Here, the 85.5% seed emergence from the control was barely above the 85% typically considered acceptable minimum germination, suggesting at this field, the one early-, one mid-season treatment was beneficial for maintaining acceptable seed emergence. One possible cause of decreased seed emergence is increased incidence of *Phomopsis* spp., which has been associated with BPMV incidence (Stuckey et al. 1982, Abney and Ploper 1994). However, seed tested for *Phomopsis* spp. from the control and from the one early followed by one mid-season application was negative, indicating *Phomopsis* spp. was not the likely cause of the decreased seed emergence.

Bean leaf beetle vector suppression early and at mid-season resulted in decreased transmission of BPMV as observed at the northwestern Iowa site in 2000. It is possible that better evidence of BPMV incidence reduction might have been achieved if more plant samples had been taken on each sample date and on more dates throughout the season. Additionally, beetle suppression in the twice early-, and one early-, one mid-season treatments at the 2000 northwestern field also resulted in higher yields and better seed quality. Because the grower knew that bean leaf beetle populations were high in this field and evidence of high BPMV incidence had been suspected for several years, early insecticide intervention probably kept the bean leaf beetle population low enough to ensure the success of the treatments. It is also possible that the variety we used at this location also may have been particularly susceptible to disease.

At other locations, the main benefit conferred by the treatments was a reduction in the percentage of mottled seed, which is an important measure of seed value. In 2001, it is possible that there were fewer treatment effects because fields were planted later and beetle populations were lower. The difference in beetle populations in the two years reflects an important consideration for management, because treatments may not provide an economic benefit if beetle populations are low. In future studies, it would be helpful to determine a beetle population threshold at which beetle transmission of BPMV would not result in economic loss. A threshold of 40% BPMV infection was calculated as the minimum required for economic loss (Horn et al. 1973); however, the corresponding beetle population required to reach that threshold is not known. Additionally, because the later planted fields in 2001 had fewer beetles, future studies also could explore the possibility of combining late planting with a mid-season insecticide treatment as a management option.

No previous study has suggested or tested an early- and mid-season application management regime to reduce bean leaf beetle populations and apparent transmission of BPMV. Although the results of this study suggest it is a useful strategy, the tactics tested here should be considered short-term, transitional options for fields with a history of BPMV and large beetle populations. Long-term, sustainable options for BPMV suppression (e.g., host plant resistance) require continued exploration because sustained use of insecticide will likely result in beetle resistance.

Acknowledgments

We thank the Iowa Soybean Promotion Board and the North Central Soybean Research Program for funding this research. We thank L. Henn, D. Linn, and L. Rossiter for collaborating on this project and providing agronomic expertise at each farm. Additionally, we thank X. B. Yang for assistance with disease progress curves, R. D. Landes and R. J. Beaver for providing statistical consultation, and J. L. Todd for editing this manuscript. This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 2428, was supported by Hatch Act and State of Iowa funds.

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Received for publication 3 January 2003; accepted 14 October 2003.