id plagues of locusts. Oeco-

ew of the biological control
ustralia and Australian New
Institute of Biological Con-
ication 1, Australia.
ntrol of weeds. Annu. Rev.

C. Moran. 1982. Ecology
us weeds in South Africa. S.

Moran & J. H. Hoffmann.
as determinants of the pres-
ndance of invasive cacti in
4. *In* I. A. W. Macdonald,
rar [eds.], The ecology and
l invasions in southern Af-
ress, Cape Town, South Af-

5 December 1988; accepted

Biological Activity of *Beauveria bassiana* Against *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae) on Leaf Substrates and Soil

J. M. McDOWELL, J. E. FUNDERBURK, D. G. BOUCIAS,[1] M. E. GILREATH,[2] AND R. E. LYNCH[3]

North Florida Research and Education Center, University of Florida, Quincy, Florida 32351

Environ. Entomol. 19(1): 137–141 (1990)

ABSTRACT *Beauveria bassiana* (Balsamo) Vuillimen has been identified as a naturally occurring pathogen of larval lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). Studies were conducted to determine the effects of a commercial *B. bassiana* spore preparation (ABG-6178, Abbott Laboratories) on larvae of *E. lignosellus*. *B. bassiana* was virulent to first and third instars in bioassays in which inoculum was applied to leaf substrates. First instars were more susceptible than third instars. Larvae treated with *B. bassiana* continued to develop and consume food at normal rates until they died or pupated. Higher conidial levels were required to cause mortality when inoculum was mixed into the larval soil habitat. Sterilizing soil before bioassaying resulted in a 10- and 1,000-fold reduction in LC_{50} values required to kill first and third instars, respectively.

KEY WORDS Insecta, insect pathogens, biological control, *Elasmopalpus lignosellus*

LESSER CORNSTALK BORER, *Elasmopalpus lignosellus* (Zeller), is a pest of many important agricultural crops. An alternative to chemical control for *E. lignosellus* is biological control; surveys of parasites and pathogens have revealed an array of potential biotic control agents (Chalfant et al. 1982). However, no research has been done to increase the effectiveness of these natural control agents. Pathogens identified from larvae of *E. lignosellus* include a granulosis virus; an entomopoxvirus; a microsporidium; a *Bacillus* sp.; and several fungi, including *Beauveria bassiana* (Balsamo) Vuillimen, *Aspergillus* spp., and *Fusarium* sp. (Johnson 1978, Funderburk et al. 1984). The entomopoxvirus, the granulosis virus, and the microsporidium are obligate intracellular pathogens and must be produced within larvae. In contrast, the fungal pathogen *B. bassiana* may be readily mass produced on various mycological media. This fungus, already in wide-scale use in eastern Europe, USSR, and China, is currently being researched in the United States for its potential against various soil-dwelling and stem-boring insect pests (Gottwald & Tedders 1983, Feng et al. 1985, McCoy et al. 1985).

Preliminary bioassays in our laboratory demonstrated that first instars of *E. lignosellus* were susceptible to a commercial *B. bassiana* spore preparation (ABG-6178; Abbott Laboratories, North Chicago, Ill.). The objective of our study was t determine the effects of this *B. bassiana* strain o the consumption, development, and mortality o first and third instars of *E. lignosellus*. Additiona assays also were performed to evaluate the effec of *B. bassiana*-treated soil on the survival of larvae

Materials and Methods

Elasmopalpus lignosellus were reared on an ar tificial diet (Chalfant 1975) in 30-ml plastic rearing cups at 27 ± 2°C with a 14:10 (L:D) photoperioc and 60 ± 5% RH. Larvae used in the experiments were first and third instars. Third instars used in the bioassays were transferred as neonates to excised soybean leaflets and allowed to feed until reaching the third instar.

A wettable-powder spore preparation of *B. bassiana* (batch #16297-147, Abbott Laboratories) was suspended and diluted in sterile distilled water to obtain the following range of concentrations: 0, 10, 50, 100, 500, and 1,000 colony-forming units (CFU)/cm² leaf surface. A 40-μl droplet of each concentration was spread across the underneath surface of a soybean leaf disk (1.7 cm diameter) and allowed to air dry. One first or third instar was placed in each 30-ml plastic rearing cup containing one treated leaf disk and allowed to feed on the treated leaf disk for 5 d. Cups were placed in plastic crispers and maintained in an incubator at 27 ± 2°C and photoperiod of 14:10 (L:D). The experiment consisted of six treatments with three replications per treatment and 20 larvae per replication. Mortality was monitored 2, 5, 7, 9, 12, 14, 16, 19, and 21 d following treatment. Untreated control

[1] Department of Entomology and Nematology, University of Florida, Gainesville, Fla. 32351
[2] Florida Department of Agriculture and Consumer Services, Tallahassee, Fla. 32399
[3] Insect Biology and Population Management Laboratory, USDA-ARS, Tifton, Ga. 31793

0046-225X/90/0137-0141$02.00/0

Table 1. Estimates of LC_{10}, LC_{50}, and LC_{90} (CFU/cm² leaf surface) for *B. bassiana* 12 d after treatment against first and third instars of *E. lignosellus*

Instar	LC (±95% CL)	Slope
First	$LC_{10} = 8.70 \times 10^{-1}$ (2.22×10^{0}–1.78×10^{-1})	1.12
	$LC_{50} = 1.21 \times 10^{4}$ (1.93×10^{4}–6.05×10^{0})	
	$LC_{90} = 1.68 \times 10^{8}$ (3.18×10^{8}–1.38×10^{8})	
Third	$LC_{10} = 1.38 \times 10^{-1}$ (1.1×10^{0}–1.06×10^{-3})	0.50
	$LC_{50} = 5.07 \times 10^{4}$ (1.82×10^{5}–1.85×10^{4})	
	$LC_{90} = 1.87 \times 10^{9}$ (8.03×10^{9}–3.73×10^{8})	

Table 2. Calculated LT_{50} values (days) for first and third instars of *E. lignosellus* treated with various concentrations (CFU/cm² leaf surface) of *B. bassiana*

CFU/cm²	LT_{50} (±95% CL)	
	First instar	Third instar
10	14.3 (17.3–12.2)	19.6 (31.5–15.7)
50	8.5 (9.7–7.2)	12.4 (15.7–10.6)
100	6.6 (7.9–4.9)	12.6 (14.5–11.4)
500	5.7 (6.3–5.0)	10.2 (12.0–8.7)
1,000	4.4 (5.1–3.5)	6.3 (8.1–3.3)

mortality data were corrected using an Abbott's (1925) formula before probit analysis was done, and estimates of LC_{10}, LC_{50}, LC_{90}, and LT_{50} and the confidence limits (CL) of each estimate were determined by probit analyses using SAS (SAS Institute 1982).

The relative consumption and development of *B. bassiana*-infected larvae were compared with those of untreated larvae. Leaf disks, removed from bioassay containers, were cleaned of debris and oven dried. Fresh leaf disks were supplied at 5, 7, 12, 14, 16, and 19 d after exposure. Consumption was measured by subtracting the weight of remaining dried leaf disks from an average of 20 whole leaf disks. Weight of surviving larvae was recorded at 9, 14, and 21 d for larvae exposed as first instar and at 7 and 14 d for larvae exposed as third instar. The stage of development of treated and untreated larvae was recorded at 7, 14, and 21 d after treatment for first instars and at 7 and 14 d for third instars. Days to pupation and pupal weight also were determined. These data were subjected to analysis of variance for a randomized complete block design (Cochran & Cox 1957) with significantly different means ($P < 0.05$) separated by Duncan's (1955) multiple-range test.

The persistence of *B. bassiana* in soil (Grossarenic Paleudult, loamy siliceous, thermic) collected in Quincy, Fla., was investigated using the same spore preparation of *B. bassiana* as described previously. The range of concentrations in this experiment was 1,000–500,000 CFU/cm³ of field soil. The experiment for first instars consisted of five fungal treatments and two soil treatments (autoclaved and unautoclaved soil), with six replications per soil treatment and 10 larvae per replication. The experiment involving third instars consisted of six fungal treatments and two soil treatments, with four replications per fungal–soil treatment and 10 larvae per replication. The autoclaved soil used in the experiments was autoclaved twice. The sorghum seedlings used in these assays were germinated in 14-cm Petri dishes lined with filter paper and kept moist with a 0.25% benomyl solution to prevent fungal growth. A 1-cm³ sample of autoclaved or unautoclaved soil was placed in a 30-ml plastic rearing cup, and a 40-μl droplet of the appropriate spore concentration was mixed gently into the soil and allowed to dry. One 5-d-old sorghum seedling and one first instar were placed in each cup containing soil treatments. Cups were placed in crispers and maintained in an incubator at 27 ± 2°C and photoperiod of 14:10. For the third instar, a second sorghum seedling was added to each cup 4 d later. Mortality of first and third instars was recorded 8 d after exposure, and dose–response data were analyzed as previously described.

Results and Discussion

Differences in susceptibility to *B. bassiana* applied on leaf substrates between first and third instars of *E. lignosellus* are evident in the analyses of the dose–response data at 12 d after treatment (Table 1). The estimated LC_{50} and LC_{90} values for first and third instars represented a 4- and 111-fold difference in susceptibility, respectively. As *B. bassiana* concentrations increased, calculated LT_{50} values decreased for first and third instars (Table 2). At dosages of 50 CFU/cm² leaf surface or higher, time to 50% mortality of first instars ranged between about 4 and 8 d, whereas 50% mortality of third instars occurred between about 6 and 12 d after treatment.

Weight and development times of untreated and surviving *B. bassiana*-treated *E. lignosellus* larvae were statistically similar (Table 3). Most larvae exposed as first instars to rates of 10^2 conidia/cm² or greater died before successfully pupating. Larvae exposed as first instars to rates of 50 conidia/cm² or less were fifth or sixth instars 14 d after exposure, with mean days to pupation at these rates ranging between 16.1 and 17.5. Mean days to pupation for larvae exposed as third instars and surviving to pupation ranged between 8.8 and 10.7 at all dosage rates.

Mean cumulative leaf consumption of untreated and *B. bassiana*-treated larvae was statistically similar (Table 4). As with estimates for weight and development, reliable estimates of cumulative leaf consumption 14 d after exposure were not obtained for larvae exposed as first instars to rates of 10^2 conidia/cm² or greater because of high mortality. Leaf consumption increased greatly with development until larvae neared pupation. Cumulative consumption of untreated larvae over development from first instar to pupation was described by the regression equation $Y = -0.00203X + 0.000393X^2$

values (days) for first and reated with various concen- e) of *B. bassiana*

$_{50}$ (±95% CL)	
	Third instar
)	19.6 (31.5–15.7)
	12.4 (15.7–10.6)
	12.6 (14.5–11.4)
	10.2 (12.0–9.7)
	6.3 (8.1–3.3)

st instar were placed in treatments. Cups were ntained in an incubator eriod of 14:10. For the ium seedling was added rtality of first and third ter exposure, and dose- /zed as previously de-

Discussion

ility to *B. bassiana* ap- tween first and third in- evident in the analyses at 12 d after treatment LC_{50} and LC_{90} values for sented a 4- and 1.1-fold , respectively. As *B. bas-* eased, calculated LT_{50} and third instars (Table m^2 leaf surface or higher, first instars ranged be- hereas 50% mortality of ween about 6 and 12 d

it times of untreated and ted *E. lignosellus* larvae Table 3). Most larvae ex- es of 10^2 conidia/cm^2 or sfully pupating. Larvae rates of 50 conidia/cm^2 stars 14 d after exposure, m at these rates ranging an days to pupation for nstars and surviving to 6.8 and 10.7 at all dosage

onsumption of untreated vae was statistically sim- timates for weight and nates of cumulative leaf posure were not obtained t instars to rates of 10^2 cause of high mortality, ed greatly with devel- d pupation. Cumulative arvae over development n was described by the $0.00203X + 0.000393X^2$ ($P < 0.0001$; $r^2 = 0.88$), where X is days of larval development and Y is mean cumulative leaf consumption in grams.

In autoclaved soil, third instars were 1,500-fold more susceptible to *B. bassiana* than first instars (Table 5). In unautoclaved soil, only a 1.5-fold difference existed between the susceptibility of third and first instars. Furthermore, autoclaving the soil substrate resulted in a 3.4- and 72.5-fold reduction in the conidial dosage required to kill an estimated 50% of first and third instars, respectively.

Based on analyses of the dose–response data (Table 1), first instars of *E. lignosellus* appear to be more susceptible to *B. bassiana* than those of other lepidopterous pests reported in the literature. For example, Ignoffo et al. (1982), using a similar assay protocol, reported LC_{50} values of 1.39×10^2 CFU/cm^2 leaf surface of *B. bassiana* against neonate *Trichoplusia ni* (Hübner). Similarly, approximately 5×10^4 CFU/cm^2 was the calculated LC_{50} value for first instars of *Ostrinia nubilalis* (Hübner) larvae (Feng et al. 1985). These values are 10- and 5,000-fold greater, respectively, than the calculated LC_{50} (12.1 CFU/cm^2) for first instars of *E. lignosellus*. The slope value of 1.12 from the dose–response regression line for first instars of *E. lignosellus* was similar to slope values of 1.07 to 1.33 calculated for the above-mentioned insects. The results of the leaf disk bioassay indicated that with maturation, larvae of *E. lignosellus* became increasingly resistant to *B. bassiana*. A similar response has been reported with other lepidopterous defoliators challenged with *B. bassiana* (Gardner & Noblet 1979, Feng et al. 1985). Dosage and the age of host had a direct influence on the time required for *B. bassiana* to kill larvae of *E. lignosellus*. Larvae treated with *B. bassiana* continued to consume foliage and mature at a normal rate.

The results of the *B. bassiana*–soil assays indicated that high levels of spores would be required to provide control of *E. lignosellus* under field conditions. To achieve 90% mortality of first or third instars, an estimated dosage of 10^5 CFU/cm^3 of soil would be required. Potentially, localizing inoculum application to the stem of host plants would reduce the inoculum load required to suppress populations. The equivalent susceptibility of first and third instars under these conditions suggests that the larger larvae are contacting higher levels of *B. bassiana* in the soil than first instars. Whether this is related to differences in insect movement, feeding behavior, or body surface area is unknown. However, these results suggest that dosages that will control neonate larvae will effectively reduce numbers of later instars. Sterilizing soil before assay caused a 10- and 1,000-fold reduction in the LC_{50} values required to kill first and third instars, respectively. Fungistatic mechanisms such as those described by Lingg & Donaldson (1981) may be operating to inhibit the bioactivity of *B. bassiana* in our soil. Alternatively, *B. bassiana* may be growing vegetatively in autoclaved soil, resulting in in-

Table 3. Effects of *B. bassiana* on weight and development of *E. lignosellus* larvae exposed as first or third instar

CFU/cm^2	Mean stage of development[a] (±SEM)						Mean larval wt, mg (±SEM)						Mean time to pupation (±SEM)					
	First instar			Third instar			First instar			Third instar			First instar			Third instar		
	n	Day 7	*n*	Day 14	*n*	Day 7	*n*	Day 9	*n*	Day 14	*n*	Day 7	*n*	Days	*n*	Days
0	49	3.5 (0.1)	35	5.9 (0.1)	49	5.9 (0.1)	42	6.9 (0.1)	45	9 (0.8)	35	21 (1.0)	49	22 (0.9)	27	16.7 (0.4)	40	10.1 (0.3)
10	49	3.5 (0.1)	29	5.9 (0.2)	50	6.0 (0.1)	35	7.0 (0)	38	8 (0.8)	30	22 (1.2)	50	23 (0.8)	19	16.1 (0.5)	36	10.5 (0.4)
50	32	3.4 (0.2)	9	5.6 (0.2)	40	6.0 (0.1)	26	7.0 (0)	23	7 (0.9)	9	21 (2.9)	41	23 (0.8)	6	17.5 (1.0)	28	9.9 (0.4)
100	27	3.5 (0.2)		ND	47	6.1 (0.1)	25	7.0 (0)	11	7 (1.0)		ND	47	23 (0.9)		ND	28	8.8 (0.2)
500	17	3.5 (0.2)		ND	39	5.8 (0.1)	22	7.0 (0)	9	6 (1.0)		ND	38	25 (1.0)		ND	24	10.6 (0.5)
1,000	8	3.9 (0.3)		ND	22	6.0 (0)	11	7.0 (0)		ND		ND	22	26 (1.4)		ND	11	10.7 (0.6)

Means within columns are not significantly different ($P < 0.05$; Duncan's [1955] multiple range test).

[a] Numbers 1 through 6 chronologically represent instars 1 through 6, number 7 represents the pupal stage; ND, not determined because nearly all larvae died.

Table 4. Effects of *B. bassiana* on cumulative leaf consumption by larvae of *E. lignosellus* exposed as first or third instar

Instar	CFU/cm²	Mean leaf dry wt consumption, mg (±SEM)[a]					
		n	Day 5	*n*	Day 9	*n*	Day 14
First	0	54	1.6 (0.2)	45	11.3 (0.7)	32	41.2 (2.3)
	10	55	1.5 (0.3)	38	11.5 (1.1)	29	41.7 (7.8)
	50	53	1.2 (0.2)	23	9.2 (1.3)	9	32.2 (6.7)
	100	44	0.4 (0.1)	11	11.3 (1.6)		ND
	500	38	1.6 (0.2)	9	8.6 (1.6)		ND
	1,000	28	1.2 (0.4)	5	5.6 (1.3)		ND
Third	0	52	31.1 (1.9)	44	53.0 (2.8)	41	55.7 (2.6)
	10	56	28.4 (1.7)	42	52.8 (3.0)	38	56.6 (2.9)
	50	49	28.6 (2.6)	33	44.6 (4.2)	28	50.5 (4.1)
	100	55	31.1 (2.0)	32	33.9 (4.2)	28	29.6 (4.2)
	500	48	29.6 (2.6)	28	37.8 (4.9)	24	39.3 (5.3)
	1,000	43	27.7 (7.0)	15	40.8 (5.8)	11	39.6 (6.3)

Means within columns are not significantly different ($P < 0.05$; Duncan's [1955] multiple range test).
[a] ND, not determined because nearly all larvae died before 14 d.

oculum levels higher than the original CFU level added to soil substrates.

Beauvaria bassiana shows promise as a biological control agent against larvae of *E. lignosellus*. The fungus was highly virulent to first and third instars when inoculum was applied to leaf surfaces, but first instars were more susceptible than third instars. Larvae treated with *B. bassiana* continued to develop and consume food at normal rates until death or pupation occurred. Higher levels were required to cause mortality when inoculum was mixed with soil. Consequently, better results may be obtained in field efficacy experiments by applying inoculum of *B. bassiana* directly to crop structures used as food rather than to the larval soil habitat.

Acknowledgment

Abbott Laboratories provided the *B. bassiana* for these studies. The research was supported in part by special research grant 88-34135-3573 from USDA-CSRS and by cooperative agreement 58-43YK-7-0034 between the USDA-ARS and the University of Florida. Florida Agricultural Experiment Station journal series 9280, Gainesville.

References Cited

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Chalfant, R. B. 1975. A simplified technique for rearing the lesser cornstalk borer (Lepidoptera: Pyralidae). J. Ga. Entomol. Soc. 10: 33–37.

Chalfant, R. B., L. W. Morgan, M. H. Bass & H. Womack. 1982. The biology of the lesser cornstalk borer, pp. 2–20. *In* H. H. Tippins [ed.], A review of information on the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). Georgia Agricultural Experiment Station Special Publication 17, Athens.

Cochran, W. G. & G. M. Cox. 1957. Experimental designs, 2nd ed. Wiley, New York.

Duncan, D. B. 1955. Multiple range and multiple *F* tests. Biometrics 11: 1–42.

Feng, Z., R. I. Carruthers, D. W. Roberts & D. S. Robson. 1985. Age-specific dose-mortality effects of *Beauveria bassiana* (Deutermycotina: Hyphomycetes) in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). J. Invertebr. Pathol. 46: 259–264.

Funderburk, J. E., D. G. Boucias, D. C. Herzog, R. K. Sprenkel & R. E. Lynch. 1984. Parasitoids and pathogens of larval lesser cornstalk borers (Lepidoptera: Pyralidae) in northern Florida. Environ. Entomol. 13: 1319–1323.

Table 5. Estimates of LC_{10}, LC_{50}, and LC_{90} (CFU/cm³ soil) for *B. bassiana* 8 d after treatment against first and third instars of *E. lignosellus*

Instar	Soil	LC (95% CL)		Slope
First	Unautoclaved	$LC_{10} = 4.43 \times 10^2$	$(1.79 \times 10^1$–$1.40 \times 10^4)$	0.79
		$LC_{50} = 1.88 \times 10^4$	$(3.24 \times 10^3$–$7.66 \times 10^5)$	
		$LC_{90} = 7.97 \times 10^5$	$(4.17 \times 10^6$–$3.48 \times 10^8)$	
	Autoclaved	$LC_{10} = 2.81 \times 10^1$	—	0.65
		$LC_{50} = 2.55 \times 10^3$	—	
		$LC_{90} = 2.32 \times 10^5$	—	
Third	Unautoclaved	$LC_{10} = 1.94 \times 10^1$	$(2.12 \times 10^1$–$3.42 \times 10^2)$	1.20
		$LC_{50} = 1.23 \times 10^3$	$(1.90 \times 10^2$–$7.37 \times 10^3)$	
		$LC_{90} = 1.45 \times 10^5$	$(3.27 \times 10^3$–$8.26 \times 10^6)$	
	Autoclaved	$LC_{10} = 4.05 \times 10^2$	—	0.49
		$LC_{50} = 1.73 \times 10^4$	—	
		$LC_{90} = 7.38 \times 10^5$	—	

lus exposed as first or third

:M)[a]

n	Day 14
32	41.2 (2.3)
29	41.7 (7.8)
9	32.2 (6.7)
	ND
	ND
	ND
41	55.7 (2.6)
38	56.6 (2.9)
28	50.5 (4.1)
28	29.6 (4.2)
24	39.3 (5.3)
11	39.6 (6.3)

t).

es Cited

nethod for computing the cide. J. Econ. Entomol. 18:

nplified technique for rearorer (Lepidoptera: Pyrali- 10: 33–37.

in, M. H. Bass & H. Wom- of the lesser cornstalk borer, ns [ed.], A review of inforstalk borer, *Elasmopalpus* ia Agricultural Experiment n 17, Athens.

ox. 1957. Experimental lew York.

tiple range and multiple *F*

., W. Roberts & D. S. Rob- e dose-mortality effects of utermycotina: Hyphomyrn borer, *Ostrinia nubilalis* . Invertebr. Pathol. 46: 259–

ucias, D. C. Herzog, R. K. s. 1984. Parasitoids and cornstalk borers (Lepidoprn Florida. Environ. Ento-

r treatment against first and

	Slope
10^{1})	0.79
10^{2})	
10^{3})	
	0.65
10^{2})	1.20
10^{3})	
10^{4})	
	0.49

Gardner, W. A. & R. Noblet. 1979. Effects of host age, route of infection, and quantity of inoculum on the susceptibility of *Heliothis virescens*, *Spodoptera eridania*, and *S. fragiperda* to *Beauveria bassiana*. J. Ga. Entomol. Soc. 13: 214–222.

Gottwald, T. R. & W. L. Tedders. 1983. Suppression of pecan weevil (Coleoptera: Curculionidae) populations with entomopathogenic fungi. Environ. Entomol. 12: 471–474.

Ignoffo, C. M., C. Garcia, M. Kroha & T. L. Couch. 1982. Use of larvae of *Trichoplusia ni* to bioassay conidia of *Beauveria bassiana*. J. Econ. Entomol. 75: 275–276.

Johnson, S. J. 1978. The population dynamics and natural mortality of the lesser cornstalk borer, *Elasmopalpus lignosellus*, in the peanut agroecosystem and the biology of selected primary parasites. Ph.D. dissertation, Texas A&M University, College Station.

Lingg, A. J. & M. D. Donaldson. 1981. Biotic and abiotic factors affecting stability of *Beauveria bassiana* conidia in soil. J. Invertebr. Pathol. 38: 191–200.

McCoy, C. W., G. M. Beavers & C. A. Tarrant. 1985. Susceptibility of *Artipus floridanus* to different isolates of *Beauveria bassiana*. Fla. Entomol. 68: 402–409.

SAS Institute. 1982. SAS user's guide: statistics. SAS Institute, Cary, N.C.

Received for publication 19 September 1988; accepted 22 May 1989.