Plant Pathol. J. 19(2) : 123-127 (2003)

Characterization of *Melon necrotic spot virus* Isolated from Muskmelon

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(Received on January 11, 2003; Accepted on April 14, 2003)

A severe disease of muskmelon (*Cucumis melo* cv. Alsnight) grown on rockwool in a plastic house was characterized by leaf and stem necrosis followed by death of the plants. In 2001, an isolate of *Melon necrotic spot virus*-MN (MNSV-MN) of the genus *Carmovirus* was identified as the causal agent of the disease on the basis of biological reactions and nucleotide sequence analyses of coat protein (CP) gene. MNSV-MN induced necrotic local lesions on mechanically inoculated leaves and systemic necrotic spots on the upper leaves of melon cvs. Alsnight, Rui III, Party, Imperial, and Seolhang. However, the inoculated leaves of watermelon and cucumber showed only necrotic lesions. DsRNAs extracted from the melon infected with MNSV-MN were separated into three components. Molecular sizes of the dsRNAs were estimated at approximately 4.5, 1.8, and 1.6 kbp. The amplified cDNA products of CP gene for MNSV-MN by RT-PCR showed approximately 1.2 kbp. The amplified DNA was digested to three fragments by *Msp*I treatment. The cDNA of the genomic RNA of MNSV-MN was cloned and the region deduced to encode the CP was sequenced. The CP coding region, located near 3' end of the genome, consisted of 1,170 nucleotides and had the potential to encode a 390 amino acid protein. The nucleotide and amino acid sequences of MNSV-MN CP gene were 84.0-94.6% and 90.8-94.9% identical with other MNSV isolates found in the GeneBank database, respectively. This is the first report on the occurrence of MNSV in Korea.

Keywords **:** *Carmovirus*, coat protein, identification, *Melon necrotic spot virus*, muskmelon, sequence.

Melon necrotic spot virus (MNSV) is a member of the genus *Carmovirus*. This genus, together with the genera *Tombusvirus* and *Necrovirus*, composes the *Tombusviridae*, a family of plant viruses with monopartite, single-stranded RNA genomes (Van Regenmortel et al., 2000). MNSV was previously shown to share certain physico-chemical characteristics with several unclassified, small, isometric plant viruses, as well as with carmoviruses and tombusviruses (Morris and Carrington, 1988; Morris and Dodds, 1979). Nucleotide sequence data showed that these two groups of viruses were closely related (Rochon and Tremaine, 1989). In the nucleotide sequences of the genomes of two Japanese MNSV isolates, NH and NK, the open reading frames (ORFs) in both genomes encode five proteins: p29 (the pre-readthrough domain of p89), p89 (the readthrough domain of p89 identified as the putative RNA-dependent RNA polymerase), p14 (the pre-readthrough domain of p7A), p7A (the putative movement protein), and p42 (CP). Nucleotide and amino acid sequence identities of the five proteins of the two isolates were estimated at 97.4-99.5% and 97.7-100%, respectively (Ohisima et al., 2000).

MNSV was first reported in muskmelon showing necrotic spot on the leaves (Kishi, 1966). The virus had narrow natural host ranges limited almost exclusively to members of the *Cucurbitaceae* (Furuki, 1981) and was transmitted through seeds and by the soil-inhabiting fungus *Olpidium borovanus* (Campbell, 1996; Hibi, 1986). Necrotic spot disease was reported from melon in Japan (Kishi, 1966) and in California, USA (Gonzalez-Garza et al., 1979), cucumber in the Netherlands (Bos et al., 1984), and watermelon in Grace (Avgelis, 1989).

In a survey on virus diseases occurring in cucurbits in 2001, muskmelon plants were noticed to have severe leaf and stem necrosis. The isolated virus from infected muskmelon was a strain of MNSV, designated MNSV-MN. This paper presents the results of these studies.

Materials and Methods

Virus isolation and bioassay. MNSV-MN was detected from muskmelon (*Cucumis melo* cv. Alsnight) showing necrosis on the leaf and stem in a plastic house in Naju, southern region of Korea, in 2001. The virus was isolated through three single lesion transfers on the cotyledon of melon seedlings. The inoculum was prepared by grinding infected cotyledons 5 days after inoculation with mortar and pestle in 1:5 (w/v) of 0.01 M phosphate buffer, pH 7.2. After mechanical inoculation using carborundum (600 mesh), the 12 plant species belonging to 21 cucurbit cultivars were maintained for visual inspection of virus symptoms in the greenhouse for at least 3 weeks (Table 2). The plants inoculated for host ranges were grown in a greenhouse at 25 ± 2°C. Tests for systemic symptomless infection were conducted by inoculating

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extracts from upper leaves to muskmelon cotyledons. The virus has been deposited in the Plant Virus GenBank.

DsRNA analysis. Viral double-stranded RNA (dsRNA) was extracted from the leaf tissues of the muskmelon systemically infected with MNSV-MN by the procedure of Morris and Dodds (1979). DsRNA of *Rice dwarf virus* was used as molecular size marker. DsRNA was analyzed by electrophoresis through 6% polyacrylamide (arcrylamide : bisacrylamide, 29:1) slab gel containing 1×TBE (1 M Tris, 0.83 M boric acid and 10 mM EDTA, pH 8.0). The dsRNA bands were visualized by silver staining method (Bio-Rad manual).

RT-PCR amplification. Total nucleic acids were extracted from the infected leaves of muskmelon by the method of Choi et al. (1998). A pair of primers for detection of MMSV-MN was designed based on nucleotide sequence analysis of the reported MNSVs (GenBank AB044708; AB044292). The primer I (5'ATGCGTTTAACCATCGCCAT3') and the primer II (5'TAGGCGAGGTAGGCGGTTTCA3') were designed to detect and amplify the CP gene of MNSV. Reverse transcriptase (RT) reaction was carried out with one cycle at 42°C for 45 minutes and 35 cycles of PCR amplification using the step program (95°C, 45 seconds; 50°C, 60 seconds; and 72°C, 60 seconds) followed by a final extension at 72°C for 10 minutes. The amplified PCR products were digested with restriction enzyme *Msp*I (Promega Corp.) and analyzed by electrophoresis in 1.5% agarose gel.

Cloning and sequencing of CP gene of MNSV. The cDNA of the CP gene of MNSV-MN was cloned into the pGEM-T-Easy vector (Promega). The nucleotide sequence was determined using a BigDye DNA sequencing kit (Perkin-Elmer Corp., Norwalk, CT, USA) on an ABI 377 DNA sequencer (PE Applied Biosystems, Foster City, CA USA). All sequences were analyzed using the DNASTAR program (Madison, Wis., USA). Homologies of nucleotide and amino acid sequences were performed using a BLAST in the GenBank database.

Results and Discussion

Incidence of MNSV disease in muskmelon. A survey on the occurrence of MNSV in muskmelon was done in Naju and Daejun in 2001 and 2002 growing seasons. Muskmelon plants naturally infected with MNSV showed severe leaf and stem necrosis (Fig. 1). Symptoms appeared on young leaves as chlorotic spots and developed into necrotic spots, often expanding to give large necrosis. Necrotic streaks on the stem and fruit stalk appeared and later, the entire plant withered. Seasonal disease incidence is shown in Table 1. The natural disease incidence of muskmelon cv. Alsnight was very low (<1%) in 30-day-old

Table 1. Virus disease incidence on muskmelon plants showing necrotic symptoms in the plastic house, 2001 and 2002

Area[a] investigated	Day after transplanting	No. of plants[b]		% infection
		Investigated	Diseased	
Naju	15	650	6	0.9
	40	650	66	10.2
	130	650	456	70.2
Daejun	30	530	3	0.6

[a] The investigation was conducted in Naju in 2001 and in Daejun in 2002.

[b] Cultivar of muskmelon grown on rockwool in Naju and on soil culture in Daejun was Alsnight.

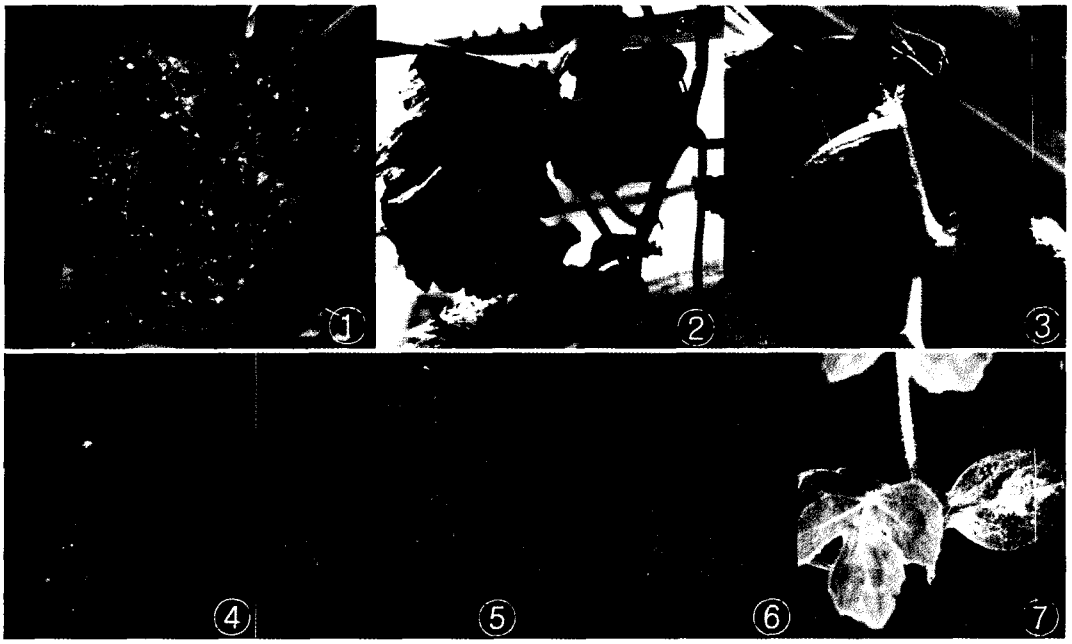

Fig. 1. Symptoms on muskmelon naturally infected with *Melon necrotic spot virus* (**1**, **2**, and **3**) and muskmelon (**4**), oriental melon (**5**), cucumber (**6**), and watermelon (**7**) mechanically inoculated with the virus.

Table 2. Biological reactions of *Melon necrotic spot virus*-MN to different test plants

Test plant	Host reaction[a]	
	Inoculated leaf	Upper leaf
Chenopodium amaranticolor	–	– (5/0)[b]
C. quinoa	–	– (5/0)
Datura stramonium	–	– (5/0)
Gomphorena globosa	–	– (5/0)
Nicotiana glutinosa	–	– (5/0)
N. clavelandii	–	– (5/0)
N. tabacum cv. Xanthi-nc	–	– (5/0)
Cucubita pepo cv. Taeyang	–	– (10/0)
C. moschata cv. Nongwooae	–	– (10/0)
Citrullus lantatus cv. Olympus	NS	– (10/0)
Cucumis sativus cv. Bagbong	CS	– (10/0)
C. melo cv. MVP	NS	NS (16/5)
cv. Best	NS	NS (14/2)
cv. Viva	NS	NS (15/8)
cv. Serhang	NS	NS (14/14)
cv. Happiness	NS	NS (24/2)
cv. Imperial	NS	NS (19/17)
cv. Saburu	NS	NS (13/13)
cv. Party	NS	NS (13/11)
cv. Sunpower	NS	NS (18/7)
cv. Bagbo	NS	NS (19/11)
cv. Olimpic	NS	NS (21/7)
cv. VIP	NS	NS (14/5)
cv. Lomans	NS	NS (22/8)
cv. Alsnight	NS	NS (34/31)
cv. Rui III	NS	NS (18/18)
cv. Salong	NS	NS (16/9)
cv. Eunsung (oriental melon)	NS	NS (72/5)

[a] Symptoms: – = no infection; NS = necrotic spot; CL = chlorotic spot.
[b] Number of plants inoculated/number of plants systemically infected

plants after transplanting in the field but was high (70%) in 130-day-old plants after transplanting. This abrupt spread of the viral disease might be due to transmission by contact between plants and/or by a vector, *Olpidium radicale* (Hibi, 1986), which has not been identified in this present experiment.

Host range. Out of 12 plant species mechanically inoculated with MNSV-MN, only three were susceptible: watermelon (*Citrullus lantatus*), cucumber (*Cucumis sativus*), and melon (*C. melo*). No symptoms were produced in *Chenopodium amaranticolor*, *C. qunoa*, *Datura stramonium*, *Gomphorena globosa*, *Nicotiana* spp., *Cucurbita pepo*, and *C. moschata* (Table 2). MNSV-MN was distinct from MNSV Cretan isolate infecting *G. globosa* (Avgelis, 1989). Melon was the only species which had systemic infection, while all other melon cultivars were not systemically infected. In oriental melon cv. Eunsung, 5 out of 73 inoculated plants produced systemic symptom while the other plants were locally infected. These results were similar to those of previous report which showed that cucumber and melon cultivars reacted severely with local

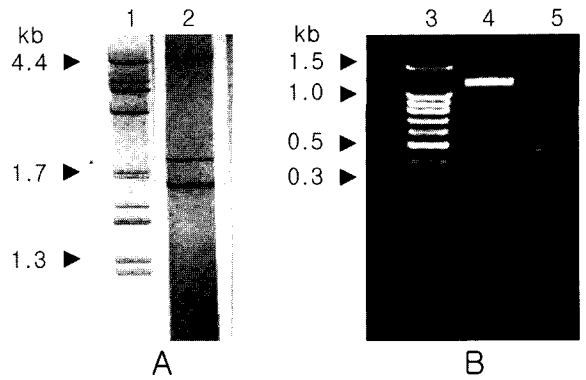

Fig. 2. DsRNA profiles of *Melon necrotic spot virus*-MN extracted from infected muskmelon (**A**) and amplified cDNA products obtained by RT-PCR with single pair of primers (**B**). Lane 1; *Rice dwarf virus* dsRNAs, 2; MNSV-MN dsRNA, 3; 1kb DNA ladder, 4: the amplified DNA products of MNSV-MN, 5: the DNAs digested with *Msp*I.

lesions, and that some even had systemic necrosis (Bos et al., 1984). It was presumed that systemic or local infection in identical cultivar will somehow be erratic under experimental conditions, or that there would be a difference in genotype resistance to the virus in each of the plants.

DsRNA analysis and RT-PCR. The dsRNAs extracted from melon infected with MNSV-MN are shown in Fig. 2A. Molecular sizes of MNSV-MN dsRNAs were estimated to be approximately 4.5, 1.8, and 1.6 kbp. Molecular size patterns of MNSV-MN dsRNAs were similar to those of the Douch isolate of MNSV (Riviere et al., 1989) and Japanese isolate of MNSV-S (Matsuo et al., 1991), while MNSV-NK and -NH (Matsuo et al., 1991) were reported to have two species of RNAs. The amplified DNA products of MNSV-MN RNA by RT-PCR showed approximately 1.2 kb, covering full-length the viral CP gene (Fig. 2B). When the amplified DNA product was digested with *Msp*I, the DNA was divided into three fragments.

Sequence analysis of CP gene of MNSV-MN. The nucleotide sequence of MNSV-MN CP gene sequence and its deduced amino acid sequence are shown in Fig. 3. MNSV-MN CP gene consisted of 1,170 nucleotides which encoded 390 amino acid residues. The sequence has been deposited in the GenBank/EMBL databases under the accession number AB106106. The identity of MNSV-MN CP gene ranged from 84.0% to 94.6% with other MNSV isolates (GeneBank accession numbers AB044708, AB044292, AF488692, AY122286, D29662, and D12536) reported previously in the nucleotide level and from 90.8% to 94.9% in the amino acid level (Table 3).

This is the first report on the occurrence of MNSV in cultivated melon plants in Korea. This report provides information on the composition of the CP gene and the biological properties of MNSV-MN for detection and identification of the virus.

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5′ ATG GCG ATG GTT AAA CGC ATT AAT AAT TTA CCA ACA GTG AAG CTT GCT AAG CAG   54
   M   A   M   V   K   R   I   N   N   L   P   T   V   K   L   A   K   Q
   GCC CTG CCC CTG CTA ACG AAT CCT AAA ATT GTG AAT AAA GCT ATA GAT GTG GTT  108
    A   L   P   L   L   T   N   P   K   I   V   N   K   A   I   D   V   V
   CCT TTG GTC GTC CAA GGT GGT CAG AAA TTG TCC AAG GCT GCC AAG CGG TTG CTT  162
    P   L   V   V   Q   G   G   Q   K   L   S   K   A   A   K   R   L   L
   GGT GCT TAT GGT GGT AAT ATT TCT TAC ACT GAG GGC GCT AAG CCA GGT GCA ATA  216
    G   A   Y   G   G   N   I   S   Y   T   E   G   A   K   P   G   A   I
   TCC GCT CCA GTT GCC ATT AGT AGG CGA GTG GCG GGT ATG AAG CCT AGG TTT GTT  270
    S   A   P   V   A   I   S   R   R   V   A   G   M   K   P   R   F   V
   CGA TCC GAA GGA TCT GTG AAG ATA GTT CAT AGG GAG TTT ATT GCC TCT GTC CTT  324
    R   S   E   G   S   V   K   I   V   H   R   E   F   I   A   S   V   L
   CCG TCG AAT GAT CTC ACC GTG AAT AAT GGT GAT GTC AAT ATC GGT AAG TAT AGG  378
    P   S   N   D   L   T   V   N   N   G   D   V   N   I   G   K   Y   R
   GTC AAT CCT AGT AAT AAT GCT TTA TTT ACA TGG CTT CAG GGA CAA GCA CAA TTA  432
    V   N   P   S   N   N   A   L   F   T   W   L   Q   G   Q   A   Q   L
   TAT GAT ATG TAC AAA TTC ACT CGG CTT CGA TTC ACC TAC ATT CCA ACT ACC GGA  486
    Y   D   M   Y   K   F   T   R   L   R   F   T   Y   I   P   T   T   G
   TCT ACT TCC ACA GGT CGT GTC TCT ATT CTC TGG GAT AGA GAC TCA CAG GAT CCC  540
    S   T   S   T   G   R   V   S   I   L   W   D   R   D   S   Q   D   P
   CTT CCT ATT GAC CGC GCT GCC ATT AGT TCT TAT GCT CAT TAC GCT GAT TCA ACG  594
    L   P   I   D   R   A   A   I   S   S   Y   A   H   Y   A   D   S   T
   CCT TGG GCA GAG AAC GTT CTA GTG GTC CCG TGT GAC AAT ACA TGG AGG TAC ATG  648
    P   W   A   E   N   V   L   V   V   P   C   D   N   T   W   R   Y   M
   AAT GAT ACC AAT GCA GTG GAC CGG AAG TTG GTC GAT TTT GGG CAG TTC CTC TTT  702
    N   D   T   N   A   V   D   R   K   L   V   D   F   G   Q   F   L   F
   GCT ACT TAT TCT GGC GCT GGT GCC ACC GCC CAT GGT GAT CTC TAC GTT GAA TAC  756
    A   T   Y   S   G   A   G   A   T   A   H   G   D   L   Y   V   E   Y
   GCC GTT GAA TTC AAA GAT CCC CAG CCT ATT GCT GGA ATG GTT TGT ATG TTT GAT  810
    A   V   E   F   K   D   P   Q   P   I   A   G   M   V   C   M   F   D
   CGC TTG GTC TCT TTC TCT GAA GTT GGA TCT ACA ATT AAA GGA GTT AAT TAT ATT  864
    R   L   V   S   F   S   E   V   G   S   T   I   K   G   V   N   Y   I
   GCC GAT CGT GAT GTG ATA ACC ACC GGG GGC AAT ATT GGT GTT CCT ATC AAT ATT  918
    A   D   R   D   V   I   T   T   G   G   N   I   G   V   P   I   N   I
   CCC GGG ACT TAT CTC GTC ACG ATT GTT CTT AAC GCT ACA TCG ATT GGC TCC CTC  972
    P   G   T   Y   L   V   T   I   V   L   N   A   T   S   I   G   S   L
   TCC TTC ACT GGC AAT TCT AAA CTC GTA GGC AAT AGT CTC AAC GTT ACC AGT AGT 1026
    S   F   T   G   N   S   K   L   V   G   N   S   L   N   V   T   S   S
   GGT GCT TCC GCT CTT ACA TTT ACC CTT AAT TCC ACT GGT GTG CCC AAC AGT ACC 1080
    G   A   S   A   L   T   F   T   L   N   S   T   G   V   P   N   S   T
   AAT TCT TCT TTT TCT GTG GGC ACC GTT GTT GCA TTG ACT AGG GTA CGT ATG GCG 1134
    N   S   S   F   S   V   G   T   V   V   A   L   T   R   V   R   M   A
   ATC ACT CGC TGC TCA CCT GAA ACC GCC TAC CTC GCC  TAA 3′                1173
    I   T   R   C   S   P   E   T   A   Y   L   A   *
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Fig. 3. Nucleotide sequences of the coat protein gene of *Melon necrotic spot virus*-MN and their deduced amino acid residues. The sequence has been deposited in the GenBank/EMBL databases under the accession number AB106106.

Table 3. Percentage sequence identities between the coat protein genes of *Melon necrotic spot virus*-MN and other MNSV isolates

MNSV source[a]	Identity (%) of coat protein	
	Nucleotide	Amino acid
AB044708	94.6	94.9
AB044292	94.5	94.6
AF488692	89.2	92.9
AY122286	84.0	90.8
D29662	93.6	94.9
D12536	89.1	91.5

[a] Homology of nucleotide and amino acid sequences of MNSV coat protein genes were performed using a BLAST in the GenBank database.

Acknowledgments

This study was supported by a grant from BioGreen 21 program, the Rural Development of Administration in Republic of Korea.

References

Avgelis, A. D. 1989. Watermelon necrosis caused by a strain of melon necrotic spot virus. *Plant Pathol.* 38:618-622.

Bos, L., Van Dost, H. J. M., Huttigna, H. and Maat, D. Z. 1984. Further characterization of melon necrotic spot virus causing severe diseases in glasshouse cucumbers in the Netherlands and its control. *Neth. J. Plant Pathol.* 90:55-69.

Choi, J. K., Kim, H. J., Hong, J. S., Kim, D. W. and Lee, S. Y. 1998. Identification and differentiation of cucumber mosaic virus isolates in Korea. *Korean J. Plant Pathol.* 14:7-12.

Compbell, R. N. 1996. Fungal transmision of plant viruses. *Annu. Rev. Phytopathol.* 34:87-108.

Hibi, T. 1986. Melon necrotic spot virus. *CMI/AAB Descriptions of Plant Viruses*, no. 302, Kew, United Kingdom.

Furuki, 1981. Epidemiological studies on melon necrotic spot. *Tech. Bull. Shizuoka Agric. Exp. Stn.* 14:1-94.

Gonzalez-Garza, R., Gumpz, D. J., Kishaba, A. N. and Bohn, G. W. 1979. Identification, seed transmission, and host range

pathogenicity of a California isolate of melon necrotic spot virus. *Phytopathology* 69:340-345.

Kishi, K. 1966. Necrotic spot of melon, a new virus disease. *Ann. Phytopath. Soc. Japan* 32:138-144.

Matsuo, K., Kameya-Iwaki, M. and Ota, T. 1991. Two new strains of melon necrotic spot virus. *Ann. Phytopath. Soc. Japan* 57:558-567.

Morris, T. J. and Carrington, J. C. 1988. Carnation mottle virus and viruses with similar properties. *In: The Plant Viruses*, vol. 3, pp. 73-112. ed. R. Koenig. Plenum Press, New York.

Morris, T. J. and Dodds, J. A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. *Phytopathology* 69:854-858.

Ohisima, K., Ando, T., Motomura, N., Matsuo, K. and Sako, N. 2000. Comparative study on genomes of two Japanese Melon necrotic spot virus isolates. *Acta Virologica* 44:309-314.

Rochon, D. M. and Tremaine, J. H. 1989. Complete nucleotide sequence of the cucumber necrosis virus genome. *Virology* 169:251-259.

Van Regenmomortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., Carstens, E., Estes, M. K., Lemon, S., Maniloff, J., Mayo, M. A., McGeoch, D., Pringle, C. R. and Wickner, R. B. 2000. Virus Taxnomy. *Seventh Report of the International Committee on Taxonomy of Viruses.* Academic Press, New York.