Almond volatiles attract neonate larvae of *Anarsia lineatella* (Zeller) (Lepidoptera: Gelechiidae)

MARK SIDNEY[1], REGINE GRIES[1], ADELA DANCI[1], GARY J.R. JUDD[2] and GERHARD GRIES[1,3]

ABSTRACT

Post-diapause overwintered larvae and neonates of any generation of the peach twig borer, *Anarsia lineatella* (Zeller), seek suitable sites to bore into and mine tissue of their host plants, including almond and peach. We tested the hypothesis that larvae are attracted to the same almond volatiles that elicit antennal responses from adult moths. Of five candidate almond semiochemicals [β-bourbonene, (*E,E*)-α-farnesene, (*E*)-β-ocimene, nonanal, decenal] tested singly or in binary combination (nonanal, decenal) in laboratory Y-tube olfactometers, only β-bourbonene attracted neonate larvae. β-Bourbonene in combination with (*E,E*)-α-farnesene was as attractive as the complete almond volatile blend, indicating that they are key semiochemicals for foraging larvae.

Key Words: Semiochemicals, β-bourbonene, (*E,E*)-α-farnesene, (*E*)-β-ocimene, nonanal, decanal, olfactometer bioassay

INTRODUCTION

The peach twig borer, *Anarsia lineatella* (Zeller) (Lepidoptera: Gelechiidae), is a worldwide pest of almond and stone fruits (Marlatt 1898, Jones 1935, Bailey 1948, Ahmad 1988, Ponomarenko 1990). Almond and peach are the principle crop host plants, but apricot, nectarine, plum and prune (Summers 1955), and even sweet and sour cherry, apple and persimmon are attacked (Ponomarenko 1990).

There are three generations of *A. lineatella* in the Okanagan Valley of British Columbia (BC) (Sarai 1966) and two generations and a partial third in the Similkameen Valley of BC. Larvae predominantly enter buds and terminal shoots, boring a path toward the centre, and then downward, often until they reach the previous year's wood (Ponomarenko 1990). In peach orchards, most economic damage is caused when larvae burrow into fruits. Larvae typically mine cavities just beneath the skin, discolouring the fruit and causing exudation of gum mixed with frass. Even when only minor damage is inflicted, cosmetic alterations reduce the fruit's value and increase picking and culling costs. Fruit damage also increases putrefaction and susceptibility to other pests (Curtis 1983). In both peach and almond orchards, severe shoot damage can stunt and kill small trees (Summers 1955).

Host-foraging and selection by *A. lineatella* is likely achieved by both female moths and larvae. Five almond-derived volatiles [β-bourbonene, (*E*,*E*)-α-farnesene, (*E*)-β-ocimene, nonanal, decanal] elicit antennal responses from female *A. lineatella* moths (Sidney 2005), and thus may be behaviourally active semiochemicals to foraging adults. When females lay eggs on sites other than a new shoot or fruit, larvae must search for a site to enter host plant tissue. Overwintered larvae emerging from hiber-

[1] Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada
[2] Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, Canada
[3] To whom correspondence should be addressed; E-mail: gries@sfu.ca

nacula may need to move considerable distances to locate new growth in spring (Bailey 1948).

Larval foraging behaviour may be mediated in part by airborne semiochemicals, as shown for codling moth, *Cydia pomonella* L. (Bradley and Suckling 1995, Landolt *et al.* 1998, Knight and Light 2001), and parsnip webworm, *Depressaria pastinacella* (Duponchel) (Carroll and Berenbaum 2002). Lepidopteran larvae can detect semiochemicals from host plants (Dethier 1980, Landolt *et al.* 1998, 2000, Singh and Mullick 2002) with semiochemical receptors residing on rudimentary antennae or maxillae (Dethier and Schoonhoven 1969, Dethier and Kuch 1971, Dethier 1980). However, such antennae are difficult to prepare for electrophysiological screening of potential host plant semiochemicals. Thus, we used adult moth antennae instead (Sidney 2005), working under the assumption that olfactory receptors are conserved across life stages, and that larval and adult antennae respond to the same semiochemicals.

Our objectives were (1) to determine whether neonate *A. lineatella* larvae orient chemo-anemotactically toward Porapak Q extracts of almond and peach shoot volatiles; and (2) if so, to determine the semiochemical(s) responsible for attraction of larvae. In this paper, we focus on bioassays with almond volatiles because β-bourbonene, the most abundant component, was present only in almonds, rendering it potentially useful for attraction of *A. lineatella* in peach orchards having no naturally occurring competing sources of the compound.

MATERIALS AND METHODS

Experimental Insects. Insects were collected from peach orchards in Keremeos, BC, and reared according to protocols developed and modified, respectively, by McElfresh and Millar (1993) and Sidney (2005).

Acquisition of Fruit and Shoot Volatiles. Freshly collected early season fruits and shoots of almond and peach were aerated separately for 3-7 d in a cylindrical Pyrex® glass chamber (15.5 × 20 cm). Charcoal-filtered air was drawn at 2 L/min with a water aspirator through the chamber and a glass column (14 × 0.40 cm ID) containing 3 cm of 50-80 mesh Porapak Q (Waters Associates Inc., Milford, MA). Volatiles were eluted from the Porapak Q with 3 ml of redistilled pentane and refrigerated (4 ˚C) until use.

Olfactometer Bioassays. Anemotactic responses of neonate larvae to test stimuli were assessed in a vertical Y-shaped Pyrex® glass olfactometer (Fig. 1) at 20 ± 3 ˚C and 35% ± 5% relative humidity. The olfactometer was placed vertically and illuminated from above with tubes of fluorescent "daylight" and "wide spectrum grow light" (Osram Sylvania Ltd., Mississauga, Ontario) because *A. lineatella* larvae are both negatively geotactic and positively phototactic. Two pieces of 20-gauge steel wire were suspended inside Y-tubes to facilitate movement of larvae (Landolt *et al.* 1998), with one piece connecting the opening of each side arm, and a linear piece suspended therefrom (Fig. 1). Visual cues were standardized by enclosing the olfactometer on three sides with black poster board.

Treatment and control test stimuli were micropipetted onto Whatman No. 2 filter paper discs (1.27 cm diameter) inserted 1 cm into the orifice of each side arm. All pipetting was done in a separate room to avoid contamination. For each replicate, a freshly-cleaned and oven-dried Y-tube with new steel wire, insect and filter paper were used, with test stimuli randomly assigned to side arms. Air drawn through the apparatus at 0.1-0.2 L/min with a water aspirator was humidified before entering the side arms. Nalgene® tubes running from the humidifiers to the side arms were dedicated as treatment or control tubes to avoid contamination. Thirty seconds after placement of stimuli, a neonate was introduced into the Y-tube on the linear piece of wire. All neo-

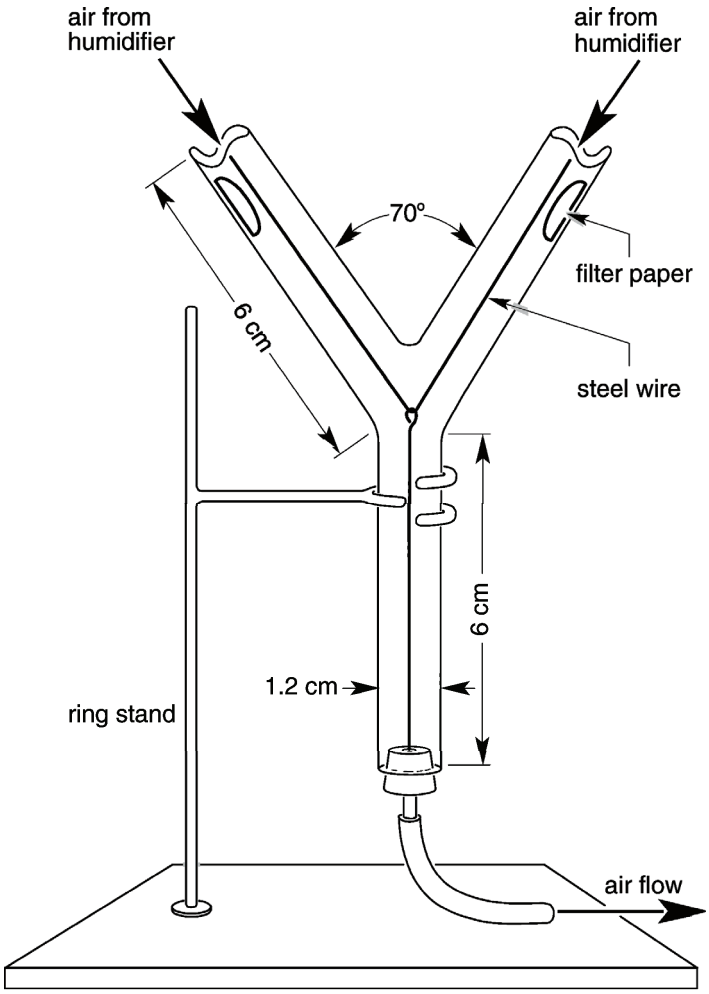

Figure 1. Vertical Y-tube olfactometer used for testing chemoanemotactic responses of neonate *Anarsia lineatella* larvae to test stimuli in experiments 1–11. Neonates were placed on the steel wire and classed as responders when they crawled > 2 cm up a side arm within 10 min; all others were classed as non-responders and were not included in statistical analyses.

nates were less than 5 h old at the time of bioassay. Neonates that travelled more than 2 cm up a side arm within 10 min were classed as responders; all others were classed as non-responders and were not included in statistical analyses.

Stimuli tested in experiments 1-11 are listed in Tables 1 and 2. Experiments 1 and 2 tested Porapak Q extract of peach and almond shoots, respectively, and experiments 3-11 tested five synthetic or plant-derived almond volatiles that elicited responses from adult moth antennae (Sidney 2005). Experiments 3-6 tested three of those five components singly, and nonanal and decanal in combination. To determine whether β-bourbonene, as the most attractive of the five components, was solely responsible for attraction of neonates to Porapak Q almond extract, experiment 7 tested β-bourbonene versus Porapak Q almond extract. Because Porapak Q extract was more attractive than β-bourbonene, experiments 8-10 tested β-bourbonene singly versus binary combinations of β-bourbonene with (*E,E*)-α-farnesene (Exp. 8), (*E*)-β-ocimene (Exp. 9), or with nonanal plus decanal (Exp. 10). Experiment 11 tested β-

Table 1.

Name, amount, chemical purity, and source of stimuli tested in experiments 1-11.

Stimuli	Amount (ng / lure)	Chemical purity (%)	Source
Porapak Q peach twig extract[1]	85.0		
Porapak Q almond twig + fruit extract[2]	39.4		
β-Bourbonene	12.0	99	Saje[3,4,5]
(*E*,*E*)-α-Farnesene	3.0	99	TCI[5,6]
(*E*)-β-Ocimene	3.0	99	IFF[5,7]
Nonanal	3.2[8]	95	Aldrich
Decanal	3.2[8]	95	Aldrich

[1] Amount is the sum of all antennally active compounds present in 5 µL of Porapak Q extract
[2] Amounts of almond volatiles used were equivalent to the amounts present in 5 µL of Porapak Q almond twig and fruit extracts.
[3] Saje, Delta, BC (geranium essential oil); absolute configuration of β-bourbonene unknown
[4] Purified by high-performance liquid chromatography
[5] Purified to 99% by preparative gas chromatography
[6] TCI = Tokyo Chemical Industry, Portland
[7] IFF = International Flavours and Fragrances, New York, NY
[8] 3.2 ng of nonanal were combined with 3.2 ng of decanal

Table 2.

Stimuli tested in Y-shaped Pyrex® glass olfactometer experiments and number of neonate larvae responding.

Experiment no.	Test stimuli		Larvae tested (n)[1]
	Treatment	Control	
1	Porapak Q peach twig extract	Pentane	175 (106)
2	Porapak Q almond twig/fruit extract	Pentane	170 (105)
3	β-bourbonene	Pentane	260 (155)
4	(*E,E*)-α-Farnesene	Pentane	60 (37)
5	(*E*)-β-Ocimene	Pentane	60 (38)
6	nonanal + decanal	Pentane	90 (59)
7	β-Bourbonene	Porapak Q extract[2]	60 (34)
8	β-Bourbonene + (*E*,*E*)-α-farnesene	β-Bourbonene	80 (58)
9	β-Bourbonene + (*E*)-β-ocimene	β-Bourbonene	60 (45)
10	β-Bourbonene + nonanal + decanal	β-Bourbonene	60 (37)
11	β-Bourbonene + (*E,E*)-α-farnesene	Porapak Q extract[2]	150 (115)

[1] Number of responding insects given in parenthesis
[2] Porapak Q almond twig/fruit extract

bourbonene plus (*E,E*)-α-farnesene (the only binary combination more attractive than β-bourbonene alone) versus Porapak Q almond extract.

Data Analysis. Data were analyzed with the χ^2 goodness-of-fit test using Yates correction for continuity to determine whether observed frequencies deviated significantly from expected frequencies, under the null hypothesis that *A. lineatella* neonate larvae did not prefer either treatment or control stimuli (Zar 1996).

RESULTS AND DISCUSSION

In Y-tube olfactometer bioassay experiments, more larvae responded to Porapak Q extracts of peach shoots ($\chi^2 = 8.2$, $P < 0.005$; Fig. 2, Exp. 1) or almond shoots and fruits ($\chi^2 = 18.9$, $P < 0.001$; Fig. 2, Exp. 2) than to solvent controls, demonstrating that *A. lineatella* neonate larvae orient chemoanemotactically to host volatiles.

Of the five almond volatiles [β-bourbonene, (*E,E*)-α-farnesene, (*E*)-β-ocimene, nonanal, decanal] that were bioassayed in experiments 3-6, only β-bourbonene was attractive to neonate larvae ($\chi^2 = 22.1$, $P < 0.001$; Fig. 2, Exp. 3). How-

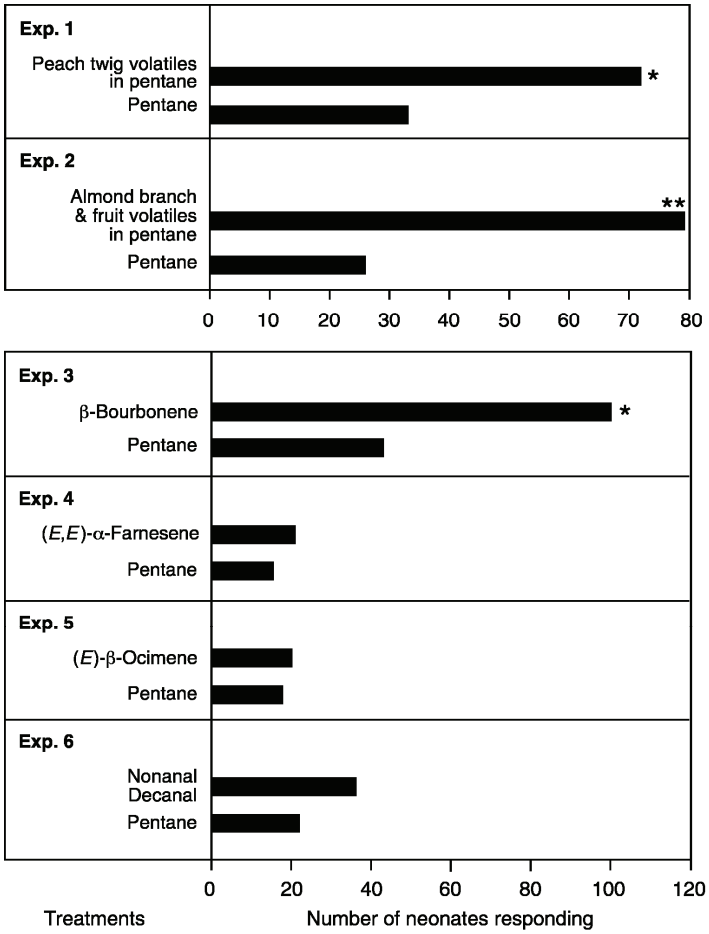

Figure 2. Anemotactic responses of neonate *Anarsia lineatella* larvae in Y-tube olfactometer experiments to Porapak Q extracts of peach twigs (Exp. 1), almond twigs and fruits (Exp. 2), or to specific candidate semiochemicals (Exps. 3-6). For each experiment, bars with asterisks (*) indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment versus control; * $P < 0.005$; ** $P < 0.001$.

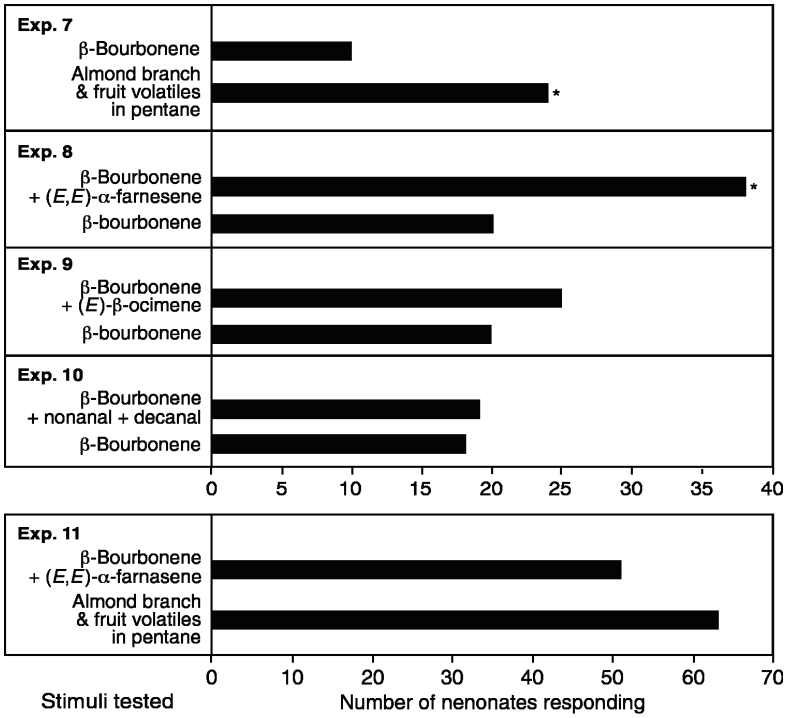

Figure 3.
Anemotactic responses of neonate *Anarsia lineatella* larvae in Y-tube olfactometer experiments 7-11 to Porapak Q extracts of almond twigs and fruits (Exp. 7), or to specific candidate semiochemicals (Exps. 8-11). For each experiment, bars with asterisks (*) indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment

ever, β-bourbonene was not as attractive as Porapak Q extracts of almond shoots and fruits ($\chi^2 = 5.4$, $P < 0.05$; Fig. 3, Exp. 7), suggesting that the almond volatiles contained at least one additional semiochemical. Of the potential synergistic components [(*E,E*)-α-farnesene, (*E*)-β-ocimene, or nonanal plus decanal] that were bioassayed in experiments 8-10, only (*E,E*)-α-farnesene enhanced attractiveness of β-bourbonene ($\chi^2 = 5.3$, $P < 0.05$; Fig. 3, Exp. 8). No difference in attractiveness between almond Porapak Q extract and β-bourbonene plus (*E,E*)-α-farnesene (Fig. 3, Exp. 11) indicated that the latter two components mediate attraction of neonate larvae to almond extract.

(*E,E*)-α-Farnesene is also a component of 'Granny Smith' apples, and attracted *C. pomonella* larvae in Petri dish bioassays (Bradley and Suckling 1995). The (*E,E*)-isomer in 'Granny Smith' apples accounts for 99.5% of the total α-farnesene content (Bradley and Suckling 1995) and it is also the predominant isomer in Porapak Q extracts of almond volatiles.

Neonate *A. lineatella* larvae seeking a feeding site are probably susceptible to predation and poor weather, as are neonate *C. pomonella* larvae under similar circumstances (Jackson and Harwood 1980). Through oriented movement toward β-bourbonene and (*E,E*)-α-farnesene, *A. lineatella* larvae would likely increase the chances of successful almond shoot or fruit location, host penetration, and survival. In integrated pest management programs for peaches, neonate *A. lineatella* could possibly be controlled by depositing bait droplets impregnated with attractive β-bourbonene and (*E,E*)-α-farnesene and laced with insecticide on tree twigs. However, this "attract and kill" tactic would be effective only if larvae were attracted over a considerable distance, and if both sesquiterpenes became commercially available.

ACKNOWLEDGEMENTS

We thank Eberhard Kiehlmann for reviewing the manuscript. The research was supported by a Research and Development grant from the Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada, and the B.C. Fruit Growers' Association to G.G. and G.J.R.J.

REFERENCES

Ahmad, T.R. 1988. Predicting emergence and flight activity of peach twig borer (Lepidoptera: Gelechiidae) by heat unit accumulation. Insect Science and its Application 9: 197-200.

Bailey, S.F. 1948. The peach twig borer. California Agricultural Experiment Station Bulletin 708, University of California, Berkeley, CA.

Bradley, S.J. and D.M. Suckling. 1995. Factors influencing codling moth larval response to α-farnesene. Entomologia Experimentalis et Applicata 75: 221-227.

Carroll, M.J. and M.R. Berenbaum. 2002. Behavioral responses of the parsnip webworm to host plant volatiles. Journal of Chemical Ecology 28: 2191-2201.

Curtis, C.E. 1983. Pest Management of Insects on Almonds. Almond Facts 38: 35-38.

Dethier, V.G. 1980. Responses of some olfactory receptors of the Eastern tent caterpillar *Malacosoma americanum* to leaves. Journal of Chemical Ecology 6: 213-220.

Dethier, V.G. and J.H. Kuch. 1971. Electro-physiological studies of gustation in lepidopterous larvae, Part 1: Comparative sensitivity to sugars, amino acids and glycosides. Zeitschrift für Vergleichende Physiologie 72: 343-363.

Dethier, V.G. and L.M. Schoonhoven. 1969. Olfactory coding by lepidopterous larvae. Entomologia Experimentalis et Applicata 12: 535-543.

Jackson, D.M. and R.F. Harwood. 1980. Survival potential of first instars of the codling moth in laboratory experiments. Annals of the Entomological Society of America 73: 160-163.

Jones, L.S. 1935. Observations of the habits and seasonal life history of *Anarsia lineatella* in California. Journal of Economic Entomology 28: 1002-1011.

Knight, A.L. and D.M. Light. 2001. Attractants from Bartlett pear for codling moth, *Cydia pomonella* (L.), larvae. Naturwissenschaften 88: 339-342.

Landolt, P.J., R.W. Hofstetter, and P.S. Chapman. 1998. Neonate codling moth larvae (Lepidoptera: Tortricidae) orient anemotactically to odor of immature apple fruit. Pan-Pacific Entomologist 74: 140-149.

Landolt, P.J., J.A. Brumley, C.L. Smithhisler, L.L. Biddick, and R.W. Hofstetter. 2000. Apple fruit infested with codling moth are more attractive to neonate codling moth larvae and possess increased amounts of (*E,E*)-alpha-farnesene. Journal of Chemical Ecology 26: 1685-1699.

Marlatt, C.L. 1898. The peach twig borer. U.S. Department of Agriculture Farmers' Bulletin 80: 1-15.

McElfresh, J.S. and J.G. Millar. 1993. Establishment and rearing of *Anarsia lineatella* (Lepidoptera: Gelechiidae) on a meridic diet. Journal of Economic Entomology 85: 1399-1404.

Ponomarenko, M.G. 1990. Review of the genus *Anarsia* Z. (Lepidoptera, Gelechiidae) of the USSR. Entomological Review 69: 102-116.

Sarai, D.S. 1966. The peach twig borer, *Anarsia lineatella* Zell. (Lepidoptera: Gelechiidae) in the Okanagan and Similkameen Valleys of British Columbia. Journal of the Entomological Society of British Columbia 63: 19-22.

Sidney, M. 2005. Stimuli affecting host-selection by larva and adult peach twig borers, *Anarsia lineatella* (Lepidoptera: Gelechiidae). Master of Science thesis, Simon Fraser University, Burnaby, Canada.

Singh, A.K. and S. Mullick. 2002. Leaf volatiles as attractants for neonate *Helicoverpa armigera* Hbn. (Lep., Noctuidae) larvae. Journal of Applied Entomology 126: 14-19.

Summers, F.M. 1955. The peach twig borer. California Agricultural Experiment Station Circular 449, University of California, Berkeley, CA.

Zar, J.H. 1996. Biostatistical Analysis, Prentice-Hall, Upper Saddle River, NJ.