Reduced Susceptibility of Organophosphate-resistant Tobacco Aphids (Homoptera: Aphididae) to Aldicarb[1]

R. M. Brierley, E. P. Lampert and C. D. Harlow

Department of Entomology
Box 7628
North Carolina State University
Raleigh, NC 27695-7628 USA

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ABSTRACT Field and greenhouse experiments were conducted to determine if organophosphate (OP)-resistant (R) and OP-susceptible (S) tobacco aphids exhibited tolerance or resistance to aldicarb, a carbamate insecticide. In the greenhouse, OP-R and OP-S aphids were placed in leaf cages on greenhouse-grown, flue-cured tobacco plants treated with various doses (0.0 to 1.0 g per plant) of aldicarb in the potting soil. Mortality was recorded after 24 h, and a dose-mortality relationship was examined. OP-S aphids were more susceptible as compared with OP-R aphids, to all doses of aldicarb. Leaf disks were collected on several dates from field-grown, flue-cured tobacco plants treated with 0, 11.2 or 22.4 kg per ha of aldicarb before transplanting. The leaf disks were taken to the laboratory, transferred to Petri dishes, and infested with ten OP-R or ten OP-S aphids. After 36 h, significantly higher mortality was observed in the OP-S aphids as compared with the OP-R aphids. These are the first test results to demonstrate a reduced susceptibility of OP-R tobacco aphids to aldicarb, a carbamate insecticide. The hypothesis that tobacco aphids with a chromosomal translocation are less susceptible to other types of insecticides is supported.

KEY WORDS Insecta, tobacco aphids, *Myzus nicotianae,* insecticide resistance.

Tobacco aphids, *Myzus nicotianae* Blackman, cause millions of dollars in damage to tobacco each year in North Carolina. Tobacco aphids are red or green in body color and exhibit varying levels of insecticide resistance (Lampert and and Dennis 1987, Harlow 1989, Abdel-Aal et al. 1990, Harlow and Lampert 1990, Harlow et al. 1991). The red color form of tobacco aphid in North Carolina was observed first at the Central Crops Research Station, Johnston Co., in 1985 and red tobacco aphids became the predominant color form in tobacco fields by 1987 (McPherson 1989, Harlow et al. 1991). Additionally, many instances of organophosphate (OP) insecticide failure against tobacco aphids were reported from 1986-1988. Laboratory experiments demonstrated an elevated level of carboxylesterase in the tobacco aphids collected where insecticide failure had occurred (Harlow 1989, Abdel-Aal et al. 1992). In later experiments, a relationship between carboxylester-hydrolyzing activity and OP resistance was shown (Harlow 1989, Abdel-Aal et al. 1990, Harlow and Lampert 1990). Recently, Wolff et al. (1994) were able to purify and characterize the resistance-associated esterase present in the OP-resistant tobacco aphids. OP-resistant

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(OP-R) tobacco aphids usually are red in body color and possess a chromosomal translocation (12T), which is linked to elevated levels of resistance-associated esterases. In contrast, OP-susceptible (S) tobacco aphids are green in body color and possess a normal (12N) chromosome configuration (Harlow 1989, Abdel-Aal et al. 1990, Harlow et al. 1991). The OP-R tobacco aphids are resistant to a wide range of commonly used organophosphate insecticides (Harlow 1989, McPherson and Bass 1990). There is concern that the OP-R aphids also may exhibit resistance to other classes of insecticides (carbamates specifically) due to similarities in the mode of action of these insecticides (Brattsten 1989). Aldicarb, a carbamate insecticide, currently provides excellent tobacco aphid control when it is incorporated into the soil before transplanting, and aldicarb is recommended for preventative aphid control in North Carolina and Virginia (Southern 1992, Semtner and Reed 1992).

The objective of this research was to determine if tolerance or cross-resistance to aldicarb exists in OP-R tobacco aphids. Specifically, two experiments were done to determine the toxicity of aldicarb to OP-R and OP-S tobacco aphids from greenhouse- and field-treated plants. In the greenhouse experiment, mortality of OP-R and OP-S tobacco aphids exposed to greenhouse-grown tobacco plants treated with varying dosages of aldicarb was determined. In the field experiment, tobacco aphids were exposed to tobacco plants treated in the field with varying dosages of aldicarb and mortality was calculated.

Materials and Methods

Aphid Source. Tobacco aphids used in these experiments were obtained from colonies maintained on tobacco plants (McNair 373) in the greenhouse at ca. 30°C with a photoperiod of 16:8 (L:D) h. Under these conditions, the average generation time is ca. 2 weeks. (Lampert and Dennis 1987). Greenhouse colonies were kept in 32-mesh lumite (Chicopee Manuf., Gainesville, GA) screen cages (1m × 2m × 1.5m). The OP-R tobacco aphid colonies were started with aphids collected from field plants in Duplin Co., NC, on 18 Sept 1986 (Harlow and Lampert 1990), and the OP-S tobacco aphid colonies were started with aphids collected from field plants at the Central Crops Research Station, Johnston Co., NC, on 23 Aug 1983 (Throne and Lampert 1985). OP-R tobacco aphids used in these experiments have been shown to exhibit resistance to several OP insecticides, possess a chromosomal translocation (12T), and have a red body color. OP-S tobacco aphids used for the study have been shown to be susceptible to OP insecticides, possess a normal chromosome configuration (12N), and have a green body color (Harlow 1989, Abdel-Aal et al. 1990, Harlow and Lampert 1990, Harlow et al. 1991).

To verify that the OP-R aphids used in our study were still resistant to OP insecticides, we used a discriminating dose test. Harlow (1989) found the LC_{50} values of malathion to be 23.9 ppm (19.4-28.2, fiducial limit) and 91.7 ppm (79.7-101.0, fiducial limit) for OP-S and OP-R tobacco aphids, respectively. Using the procedures of Harlow (1989), we prepared suspension of 0, 30, 75, and 150 ppm malathion (Malathion 50 Plus, EC, Chevron Chemical Co., San Ramon, CA.) in distilled water. Aphids were mounted, ventral side up, to double-stick cellophane tape attached to a microscope slide. Fifteen aphids were mounted on a

slide for each concentration and three replicates were used (45 aphids per concentration). After all aphids for a concentration were mounted, the slides were submerged individually in the suspension for 30 s. Slides were allowed to air dry at 23°C for 24 h, then mortality was determined. An aphid was considered dead if it did not show leg movement after being probed gently with a camel's hair brush for 10 s. For both the OP-S and OP-R colonies, mean mortality was calculated for each concentration of malathion.

Greenhouse Bioassay. Greenhouse experiments were conducted at the Method Road Complex, NCSU, Raleigh. Flue-cured tobacco plants (var. McNair 373, 4- or 5-leaf stage) were transplanted into clay pots (10.2 cm diam.) on 20 May 1991. For each plant, approximately 3 cm of potting soil (Metro-mix 220, Grace Horticultural Products, Cambridge, MA) was placed into a pot and then a pre-weighed amount of aldicarb (Temik® 15G, Rhone-Poulenc Ag Company, RTP, NC) was distributed uniformly on the potting soil surface and covered with an additional 2 cm of potting soil. This procedure is similar to recommended field applications where aldicarb is broadcast on the soil surface and covered immediately by the bed-forming process. Aldicarb was applied at 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 g, or 1.0 g per plant (field rate ≈ 0.75 g per plant). A tobacco plant was transplanted into the treated soil, and the remainder of the soil was added to the pot. Pots were transferred to the greenhouse where they were maintained at ca. 30°C and a photoperiod of 16:8 (L:D) h. Pots were placed on clay saucers, and plants were watered in the saucers to prevent leaching of the aldicarb from the treated soil.

On each test date, tobacco aphids were transferred from the OP-S or OP-R colonies to treatment plants. Ten mixed-age apterous adult tobacco aphids from each colony were transferred with a camel's-hair brush to each treated tobacco plant, where they were contained in round, plastic clip-on cages (2.5 cm diam × 1.5 cm ht) with lumite (32 mesh) covers to allow air flow. Two cages were clipped on each plant – one cage on each side of the mid-vein on the underside of a healthy leaf > 15 cm in length. Three plants were infested for each dose of aldicarb on each treatment date. At specified days after transplanting (DAT) (20 June, 31 DAT; 26 June, 37 DAT; and 16 July, 57 DAT), ten OP-R and ten OP-S mixed-age, adult tobacco aphids were collected from the standard colonies and transferred to aldicarb-treated plants where they were confined in separate leaf cages.

For each treatment, the numbers of alive and dead nymphs and adults were recorded 24 h after adult aphids were transferred to the cages. Aphids were probed with a camel's-hair brush for 10 s; if an aphid did not move after probing, it was considered dead. Using these data, the proportion of dead aphids (mortality) was calculated. The mean mortality for each dose was determined using Proc Means (SAS Institute 1985).

Field Bioassay. For the field bioassay, 0, 11.2, and 22.4 kg per ha. of aldicarb (Temik® 15G formulation per acre) were applied to treatment plots on 23 April 1991 and incorporated immediately according to label directions. Plots (two rows wide, 2.3 m, × 20 plants) were arranged according to a randomized complete-block design with four blocks. Flue-cured tobacco (McNair 373) was transplanted into the pots on 24 April 1991. Standard agronomic practices for production of flue-cured tobacco were followed (Peedin et al. 1991). On 19 June,

26 June, and 17 July (56 DAT, 63 DAT and 84 DAT, respectively), leaf disks were collected from these field plants for bioassay. Five consecutive tobacco plants from each treatment were selected randomly, and two leaf disks (50 mm diam) were cut from one leaf on each plant, one disk from each side of the midvein. The first, fully expanded leaf (> 15 cm) below the bud was selected from each plant. Leaf disks were placed on moistened filter paper (4.25 cm, Whatman 54, Whatman International Ltd., Maidstone, ENG) in Petri dishes (50 mm diam, Falcon Seal Tight, Becton Dickinson, Lincoln Park, NJ), labeled, and placed on ice in a cooler for transport to the laboratory. At the laboratory, ten OP-S and ten OP-R tobacco aphid adults were transferred from colonies to each disk using a camel's-hair brush.

To determine the optimum time to record mortality, the numbers of living and dead tobacco aphids (adults and nymphs) were recorded 12, 24, 36, 48, and 72 h after aphid transfer using the above criteria for aphid death. Mortality was calculated after each evaluation period, and it was determined that a 36h evaluation time should be used.

Thirty-six h after aphids were transferred to leaf disks, the mean numbers of surviving nymphs, adults, and total aphids, and the proportions of nymphs and adults surviving were calculated for each treatment using Proc Means (SAS Institute 1985). Proportions were transformed to arcsine $\sqrt{\text{proportion}}$ before using analysis of variance, Proc ANOVA (SAS Institute 1985), to determine treatment effects. When significant treatment effects were noted, a Waller-Duncan K-ratio t test was used to separate treatment means (SAS Institute 1985).

Results and Discussion

Tobacco aphids from the OP-R colony still exhibited resistance to malathion as compared with tobacco aphids the OP-S colony. At 30 ppm of malathion, 82.9% (SEM = 3.6, n = 3) of the OP-S tobacco aphids died as compared with only 1.9% (SEM = 2.4, n = 3) of the OP-R tobacco aphids. One hundred percent of the OP-S tobacco aphids died at malathion concentrations above 75 ppm; whereas, only 36.4% (SEM = 3.5, n = 3) and 52.5% (SEM = 1.3, n = 3) of the OP-R tobacco aphids died at malathion concentrations of 75 and 150 ppm, respectively. These results are in close agreement with those of Harlow (1989), when the OP resistance was first documented in these colonies, and would indicate that malathion resistance was still present in the OP-R colony after 6 years of rearing in the laboratory.

Greenhouse Bioassay. On 24 May 1991, phytotoxicity was observed on some of the plants treated with 0.25, 0.35, 0.50, and 1.0 g doses of aldicarb. At the lower rates (0.25-0.50 g), phytotoxic symptoms were leaf curling and small amounts of chlorosis on the leaf margins; while at the high dose (1.0 g) large patches of chlorotic tissue were observed, followed by necrosis and, in some cases, plant death. Symptoms were most severe on the leaf margins and midveins. The severity of phytotoxicity in the 1.0 g plants prevented their further use in this experiment.

On all three sample dates, mortality of the OP-S tobacco aphids was higher than that of the OP-R tobacco aphids for each dose of aldicarb (Fig. 1.) As the

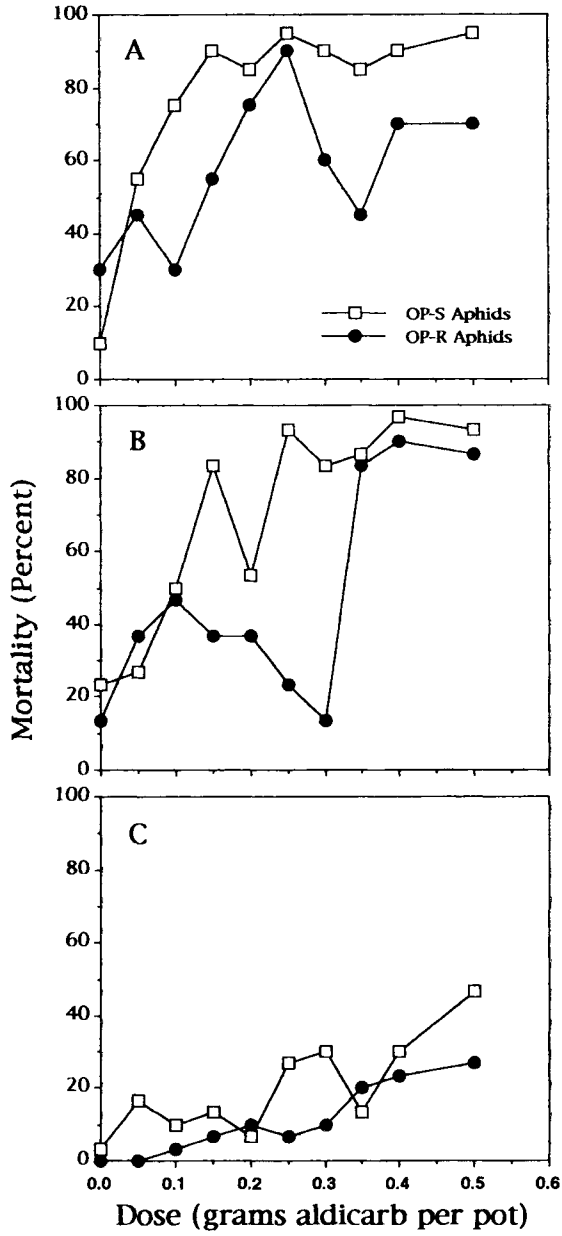

Fig. 1. Relationship between the mortality of organophosphate-susceptible (OP-S) and organophosphate-resistant (OP-R) tobacco aphids to varying doses of aldicarb applied to greenhouse-grown, flue-cured tobacco. Mortality determined (A) 20 June 1991 (31 DAT), (B) 26 June 1991 (37 DAT) and (C) 16 July 1991 (57 DAT).

time after transplant increased, a reduction in the mortality was observed. This is assumed to be related to the increased growth of the tobacco plant, which would result in a dilution of the aldicarb concentration throughout the plant tissue and the metabolism of the aldicarb residues by the plant. This test demonstrated the reduced susceptibility of OP-R tobacco aphids as compared with OP-S aphids and supported the need to conduct the field study.

Only the aphids placed on leaf disks collected from aldicarb-treated, greenhouse grown tobacco plants on 26 June 1991 produced enough nymphs to examine the effects of various doses of aldicarb on nymphal survival. Survival of OP-S and OP-R tobacco aphid nymphs decreased as the dose of aldicarb increased from 0.0 to 0.25 g per pot (Table 1). At doses beyond 0.25 g aldicarb per pot, nymphal survival did not follow a consistent trend. This is due, in part, to the low number of nymphs produced in general and the absence of nymph production by adults in some of the replicates. Nymph survival was generally higher in the cages containing OP-R aphids as compared with cages containing OP-S aphids; however, due to the low number of nymphs produced by the surviving adults, these differences were not statistically significant.

Table 1. Average survival of organophosphate susceptible (OP-S) and organophosphate resistant (OP-R) tobacco aphid nymphs after 24 h exposure to leaf disks from aldicarb-treated tobacco plants grown in the greenhouse, 26 June 1991, 37 days after treatment.

Aphid	Dose*									
Type	0	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.50
OP-S, Green										
Survival (%)	100.0	82.0	66.7	30.0	44.4	0.0	24.4	15.0	0.0	25.0
SEM, n = 3	0.0	12.8	33.3	30.0†	29.4	0.0	12.4	7.6	0.0†	25.0†
Total**	5.3	8.0	2.33	2.3	2.3	2.3	3.7	3.3	1.7	1.7
OP-R, Red										
Survival (%)	100.0	100.0	83.8	58.3	50.0	52.8	100.0	11.1	0.0	0.0‡
SEM, n = 3	0.0	0.0	16.7	8.3†	50.0†	26.5	0.0†	11.1	0.0†	0.0
Total	7.7	5.0	2.3	2.7	1.0	4.0	2.0	2.3	1.0	0

* g aldicarb (Temik 15G) per pot.
** Average total number of nymphs produced per cage.
† No nymphs produced by the adults in one replicate, survival based on n=2.
‡ No nymphs produced by the adults in any of the replicates.

Field Bioassay. As the time after aphid transfer to the aldicarb-treated leaf disks increased, mortality increased in both OP-S and OP-R tobacco aphids (Fig. 2). Between 12 and 48 h after transfer, OP-S tobacco aphids exhibited a higher mortality as compared with OP-R tobacco aphids. At 72 h after transfer over 95% of the OP-S and OP-R aphids were dead, regardless of the dose; whereas, only approximately 6.5% of the aphids transferred to the untreated leaf disks were dead. It should be noted that there was no significant mortality difference between the 11.2 and 22.4 kg rates of aldicarb in both the OP-S and OP-R tobacco aphids. Separation between OP-S and OP-R aphids confined to aldicarb-treated leaf disks was greatest at 24 and 36 h. At 36 h, mortality in both red and green aphids confined to aldicarb-treated leaf disks was much higher (>40%) than in the aphids transferred to untreated leaf disks (<10%) (Fig. 2). Based upon these results, a 36-h evaluation period was selected and used for further tests.

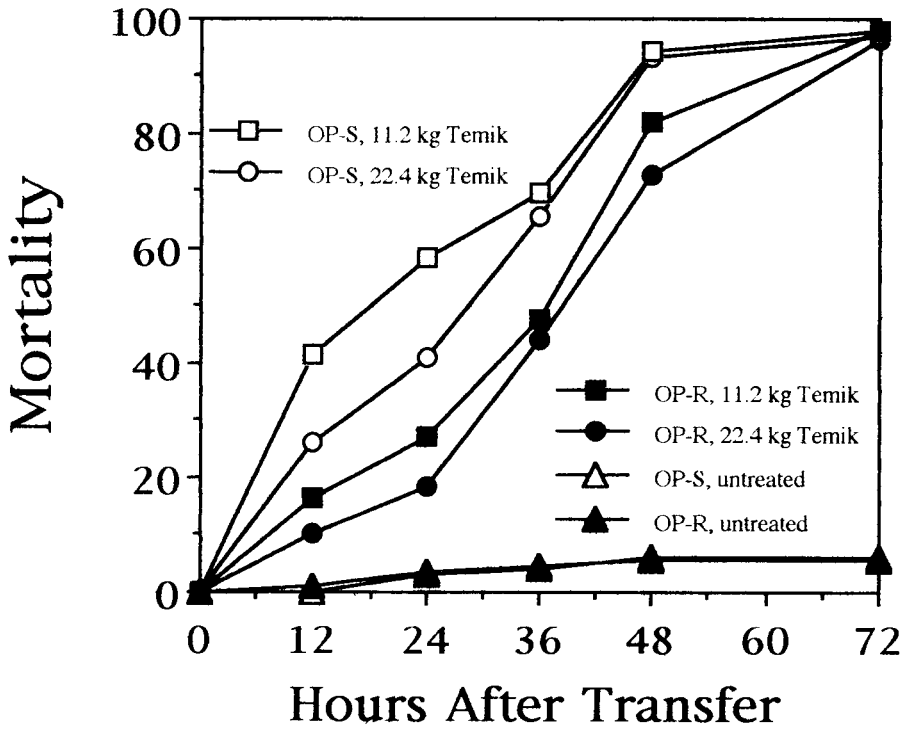

Fig. 2. Relationship between the mortality of organophosphate-susceptible (OP-S) and organophosphate-resistant (OP-R) tobacco aphids and time from aphid transfer to aldicarb-treated leaf disks. Leaf disks collected on 11 June 1991 from flue-cured tobacco treated with aldicarb on 23 April 1991, Central Crops Research Station, Johnston Co., NC.

OP-R tobacco aphids placed on leaf disks collected from field plants treated with aldicarb exhibited reduced mortality as compared with OP-S tobacco aphids placed on similar leaf disks. Significant differences in mortality were observed between the OP-S and OP-R tobacco aphids on leaf disks collected 19 June 1991 (56 DAT) (Fig. 3A; $F = 9.35$, $df = 1,22$, $P = 0.0058$). In addition, mortality was affected significantly by the dose of aldicarb ($F = 23.45$, $df = 2,22$, $P = 0.0001$). The largest difference in mortality between OP-S and OP-R tobacco aphids occurred at the 11.2 kg dose of aldicarb. As time after aldicarb application increased, the level of mortality was reduced. Significant differences in mortality were observed between the OP-S and OP-R aphids on leaf disks collected 26 June 1991 (63 DAT) (Fig. 3B; $F = 16.80$, $df = 1,22$, $P = 0.0005$); however, no significant dose effect was observed ($F = 0.48$, $df = 2,29$, $P = 0.622$). At 84 DAT (17 July 1991), the effects of the aldicarb had attenuated (Fig. 3C). There were no significant differences in mortality between the OP-R and OP-S tobacco aphids ($F = 0.12$, $df = 1,22$, $P = 0.731$) or due to the dose of aldicarb ($F = 0.72$, $df = 2,22$, $P = 0.498$) at 84 DAT. These results are in agreement with observations of Boiteau et al. (1985) who saw a reduction in the mortality of green peach aphids. *M. persicae* (Sulzer), exposed to aldicarb-treated potato plants as the days after application increased. At 43 DAT, 37.5% mortality was observed in green peach aphids exposed to aldicarb-treated potato plants as compared with on 0.5% mortality at 85 DAT. Additionally, concentrations of aldicarb residues in the potato plant were found to decrease rapidly regardless of the application rate (10, 17, or 22 kg per ha) or method of application (see furrow, fertilizer band, or top dress).

OP-R nymphs had a significantly higher survival as compared with OP-S tobacco aphids following exposure to leaf disks collected from aldicarb-treated, field-grown tobacco plants on 19 June 1991 (56 DAT) (Table 2). In addition, a significant dose effect was found in which survival decreased as the dose of aldicarb increased. Similar to adults described above, there was no significant difference in mortality between the OP-R and OP-S tobacco aphid nymphs exposed to the leaf disks collected on 26 June (63 DAT). In this case, there was no effect on nymph mortality due to increasing the dose of aldicarb.

These studies indicate that OP-R tobacco aphids are more difficult to control and have significantly lower mortality as compared with OP-S tobacco aphids when exposed to aldicarb, a carbamate insecticide. Lower mortalities in greenhouse assays and lower mortalities in field tobacco studies were observed in OP-R tobacco aphids as compared with OP-S tobacco aphids. This is in agreement with other field and laboratory studies that have shown that red tobacco aphids with translocated chromosomes are more difficult to control with OP insecticides as compared with the green, untranslocated tobacco aphids. (Abdel-Aal et al. 1990, Harlow 1989, Harlow and Lampert 1990, McPherson and Bass 1990). These two experiments document the presence of increased tolerance to aldicarb in OP-R tobacco aphids. OP-R tobacco aphids remain readily controlled by the use of aldicarb; however, because we have documented carbamate resistance, the use of aldicarb should be studied carefully so that strategies can be developed to prevent and/or retard further carbamate resistance in OP-R tobacco aphids.

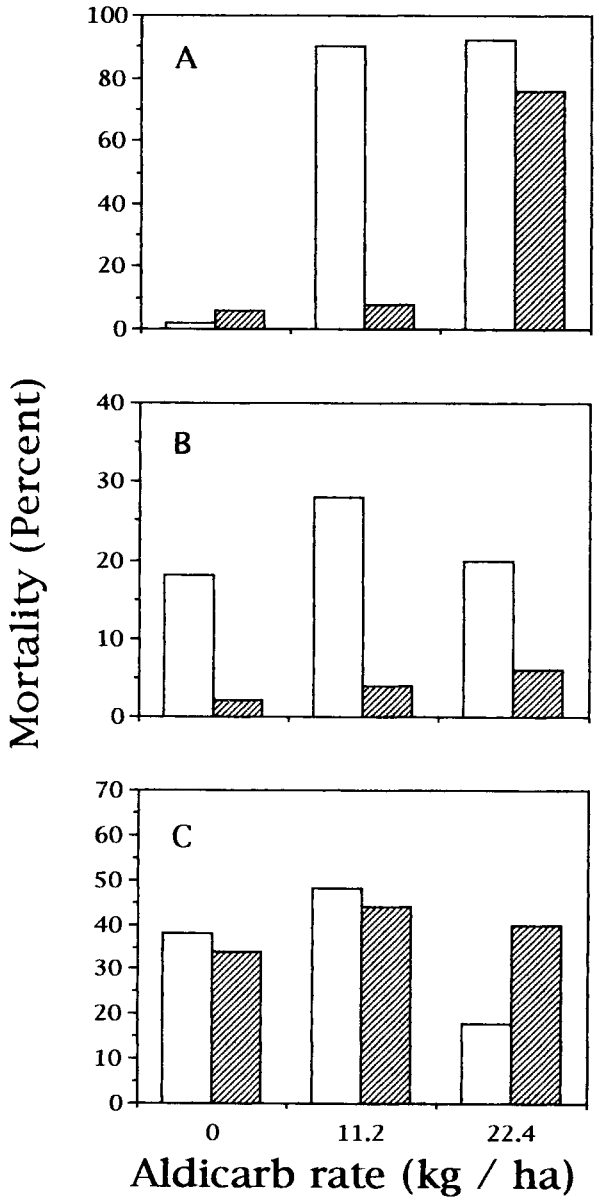

Fig. 3. Mortality of organophosphate-susceptible (OP-S) and organophosphate-resistant (OP-R) tobacco aphids exposed to leaf disks collected from field treated flue-cured tobacco. Leaf disks collected on (A) 19 June 1991 (56 DAT), (B) 26 June 1991 (63 DAT) and (C) 17 July 1991 (84 DAT) from flue-cured tobacco treated with aldicarb on 23 April 1991, Central Crops Research Station, Johnston Co., NC.

Table 2. Average survival of organophosphate susceptible (OP-S) and organophosphate resistant (OP-R) tobacco aphid nymphs after 36 h exposure to leaf disks from aldicarb-treated tobacco plants grown in the field, Central Crops Research Station, 1991.

Aphid Type	Dose* 19 June ** 0	11.2	22.4	26 June † 0	11.2	22.4
OP-S, Green						
Survival (%)	95.1	55.8	19.3	93.3	88.3	83.3
SEM, n = 5	2.0	11.5	13.9	7.3	7.3	10.5
Total nymphs‡	11.4	6.4	6.6	5.2	5.0	6.0
OP-R, Red						
Survival (%)	100.0	73.5	53.8	100.0	93.5	93.0
SEM, n = 5	0.0	14.3§	11.4§	0.0	4.2	4.9
Total nymphs	4.2	6.0	3.2	8.6	8.2	7.2

Percents transformed to arcsine $\sqrt{\text{percent}}/100$ before ANOVA (SAS Institute1985)
* kg aldicarb (Temik 15G) per ha.
** Color: F = 10.74, df = 1,20, ***P*** = 0.004; Dose: F = 27.35, df = 2,20, ***P*** = 0.0001.
† Color: F = 1.02, df = 1,22, ***P*** = 0.324; Dose: F = 1.05, df = 2,22, ***P*** = 0.366.
‡ Average total number of nymphs produced per cage.
§ No nymphs produced by the adults in one replicate, survival based on n=4.

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