Responses of apple maggot (Diptera: Tephritidae) to ammonium hydroxide lures

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The Canadian Entomologist **136**: 139 – 142 (2004)

Ammonia has long been known to be an attractant for the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Hodson 1943, 1948). The responses of apple maggot to ammonia release rates, however, have not been adequately determined. Ammonia release rates determined from mass losses of ammonium carbonate, ammonium acetate, and casein hydrolysate lures (*e.g.*, Jones 1988; Reynolds and Prokopy 1997) do not take into account decomposition and release of compounds such as carbon dioxide or volatiles that may be moderately repellent (Bateman and Morton 1981; Mazor *et al.* 1987) or attractive to some Tephritidae flies (Mazor *et al.* 1987). In this study, the objectives were to determine the responses of apple maggot females and males in western Washington to ammonium hydroxide lures designed to release a wide range of ammonia. An emphasis was placed on identifying the ammonia release rates that elicit maximum fly responses.

Study sites chosen in western Washington were based on their availability (permission to trap), their fly population levels, seasonal fly presence, and tree numbers. No sites were treated with insecticides. Sites 1 (47°11.76′N, 122°16.50′W) and 2 (47°11.78′N, 122°18.80′W) were a commercial orchard and backyard/cow pasture, respectively. Site 3 (45°53.54′N, 122°45.23′W) was a mixed apple/fruit orchard. Sites 4 (45°56.39′N, 122°40.41′W) and 5 (45°37.45′N, 122°39.78′W) were a horse pasture and a national park, respectively. Site 6 (45°35.88′N, 122°07.03′W) was an old homestead. Apple trees (many varieties) within sites were of similar size, but they varied from 2 to 11 m in height among sites. Tree spacing at site 1 was 3 m and at other sites was 10–30 m.

Lures used in all experiments were constructed from 15-mL polypropylene narrow-mouth Nalgene® bottles (Nalge Nunc International, Rochester, New York) containing 0.75 g of cotton saturated with 10 mL of ammonium hydroxide (Fisher Chem, Fair Lawn, New Jersey) solutions. A lure was hung 1–2.5 cm above a 14 cm × 23 cm sticky yellow panel (Scenturion, Clinton, Washington) in all experiments. In addition, a 7.5 cm diameter sticky red sphere (Scenturion, Clinton, Washington) was included in two experiments (see below). One trap was hung 1.5–2 m above ground on the south side of one apple tree with a moderate to heavy fruit load. Traps in a randomized block design (a block or replicate was a row or cluster of trees; n = 3–5 replicates) were repositioned daily. Traps were hung 3–10 m (sites 1–3) or 10–30 m (sites 4–6) apart. All tests were conducted for 4 consecutive days. Flies were removed daily and sexed in the laboratory.

Ammonia concentrations of 0, 1.9 ± 0.1, 3.6 ± 0.2, 7.3 ± 0.2, 14.6 ± 0.3, 20.8 ± 0.4, and 29.3 ± 0.2% (mean ± range) (29.3% is the maximum in 100% ammonium hydroxide) were tested with yellow panels. Ammonia was released through a 0.5 mm diameter hole in the bottle cap, and was not replaced during the 4 days. In 2001, tests were run for 4 weeks at sites 1, 4, and 6 (20 August – 21 September). In 2002, tests

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were run for 7 weeks at sites 1, 2, 3, 5, and 6 (15 July – 20 September). Five replicates of all concentrations were included in each test.

To test responses to higher ammonia release rates from both yellow panels and red spheres, multiple bottles and bottles with different-sized holes were tested. One bottle containing 29.3% ammonia and with a 0.5 mm diameter hole was compared with three and six such bottles. To control for possible visual effects, there were six bottles for all treatments (control: six empty bottles; one bottle treatment: one filled plus five empty; three bottle treatment: three filled plus three empty; six bottle treatment: all filled) taped to form one bundle. Ammonia was not replaced during the 4 days. In 2002, two tests were run over 8 days (between 6 and 17 August) at sites 1 (n = 5) and 3 (n = 4). In another experiment, single bottles containing 29.3% ammonia and with 0-, 0.25-, 0.5-, 1.6-, 3.2-, and 6.4-mm holes were compared. Not all sizes were included in each test. Ammonia was replaced after 2 days. Six tests were run, one each at sites 1 (n = 4) and 5 (n = 3) (0-, 0.5-, and 1.6-mm sizes tested) for 4 days (30 July – 4 August 2001); one each at sites 1 (n = 4) and 5 (n = 3) (0-, 0.5-, and 3.2-mm sizes tested) for 12 days (19 July – 17 August 2001); and one each at sites 1 (n = 5) and 3 (n = 5) (0-, 0.25-, 0.5-, and 6.4-mm sizes tested) for 16 days (24 July – 7 September 2002).

Ammonia release rates from bottles with different ammonia concentrations were determined at days 0 and 4, and from those with different holes at days 0 and 2 in the laboratory at 21 °C using the Nesslerization method (Rana and Mastrorilli 1998) (six of each lure). Between collections, lures with different concentrations were aged in a fume hood (constant airflow of 25.3 m/min, 19.5 °C (mean), and 30–40% RH), as were lures with different-sized holes (airflow of 36.9 m/min (11 h) and 7.9 m/min (13 h), same temperature and RH).

Data (after square-root transformation) from the ammonia concentration experiment were subjected to factorial ANOVA (factors were ammonia concentration, site, study week, and block). Quadratic regression was also performed on the mean numbers of flies/trap per day. Data from bottle-number and hole-size experiments were subjected to ANOVA, followed by Tukey's test to discriminate the means (SAS Institute Inc 2001).

For both males and females in 2002, fly catches on yellow panels revealed effects (P < 0.004) of ammonia concentration ($F_{6,332}$ = 9.90 (males), 14.52 (females)), site ($F_{3,332}$ = 4.47 (males), 14.18 (females)), week ($F_{7,332}$ = 9.77 (males), 20.29 (females)), and block ($F_{4,332}$ = 5.64 (males), 6.14 (females)), but no interactions (P > 0.06) between concentration and site ($F_{18,332}$ = 1.45 (males), 1.59 (females)) or concentration and week ($F_{42,332}$ = 1.26 (males), 1.17 (females)). This same pattern was observed in 2001 with ammonia concentration ($F_{6,192}$ = 2.62 (males), 8.95 (females)) affecting catch (P < 0.02), except that no effects of site, week (except for females), block, or interactions were detected (P > 0.06). Both sexes had weak responses to 1.9% ammonia. Females had strong responses to 20.8% and 29.3% ammonia and males had strong responses to 14.6%–29.3% ammonia (Fig. 1). In both years (all sites), 65% of flies on baited panels were female (n = 2589).

In two tests at two sites, traps with one, three, and six baited bottles caught similar numbers of flies (Tukey's test, P > 0.05). Differences between controls and treatments for a trap type differed at site 3 but not at site 1. Spheres usually caught more flies than panels, but no consistent differences were detected between trap types, particularly when traps were baited. Females predominated on baited panels (57%, n = 746), but males predominated on baited spheres (58%, n = 1291).

In six tests at three sites, traps releasing ammonia from different-sized holes consistently collected more flies of either sex than unbaited traps for both yellow panels and red spheres. For treatments with bottles having the same orifices, red spheres collected more males in all six tests and more females in five of six tests than yellow

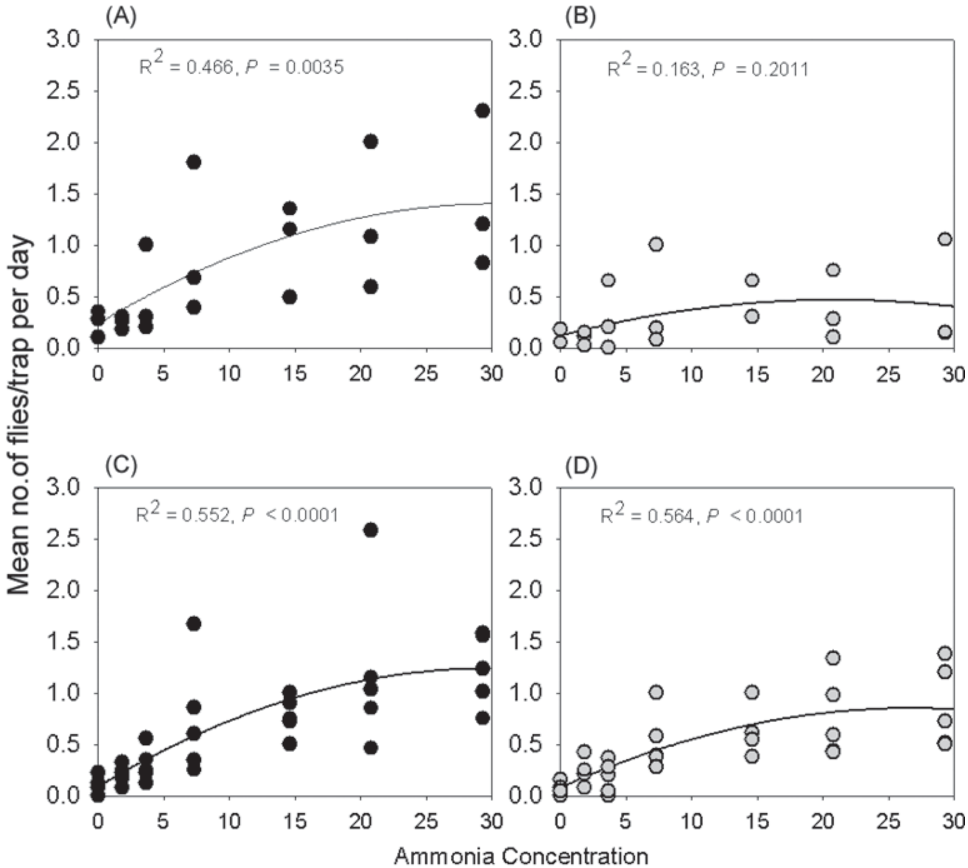

FIGURE 1. Responses of *Rhagoletis pomonella* females (A, C) and males (B, D) to sticky yellow panels baited with different ammonia concentrations in 2001 (*n* = 3 sites; A, B) and 2002 (*n* = 5 sites; C, D) in Washington. Each point within a concentration represents cumulative seasonal data from 1 site and 5 replicates.

panels. For either trap type, no effect of dispenser hole size was detected (Tukey's test, $P > 0.05$). Catches ranged from 0.2 to 4.6 flies/day per baited yellow panel and from 0.9 to 5.1 flies/day per baited red sphere. Flies on baited panels (72%, $n = 2537$) and spheres (57%, $n = 4166$) were mostly female.

Release rates increased with greater ammonia concentrations (day 0, $y = 0.105–0.025x + 0.006x^2$, $R^2 = 0.984$, $P < 0.0001$; day 4, $y = 0.073–0.003x + 0.004x^2$, $R^2 = 0.979$, $P < 0.0001$) and hole sizes (day 0, $y = 0.765 + 57.006x + 153.294x^2$, $R^2 = 0.995$, $P < 0.0001$; day 2, $y = 1.389 + 17.096x + 84.755x^2$, $R^2 = 0.984$, $P < 0.0001$). These rates ranged from 0.06 to 99.9 mg ammonia/bottle per h (mg/h). Flies responded weakly to the lowest ammonia concentration, which released 0.06 mg/h, suggesting this rate may be near the lower threshold of detection. Treatments that elicited maximum responses released 1.9–4.7 mg/h (day 0) and 1.4–3.5 mg/h (day 4).

These results indicated that apple maggot females and males responded somewhat differently to ammonia and trap type. Responses of females to higher ammonia release rates from yellow panels and red spheres were greater than that of males, perhaps because females needed more protein than males (Webster and Stoffolano 1978). Females responded more to panels than did males, perhaps because panels better mimic leaves that have proteinaceous food than fruit (Prokopy 1968).

Several other relationships between ammonia lures and trap type on fly responses were noteworthy. One was that baited panels and control spheres performed similarly, but baited spheres captured more flies. Baited spheres were also more effective than control spheres in half the tests (and numerically more so in all). Other studies (Reynolds and Prokopy 1997; Rull and Prokopy 2000; Stenliski and Liburd 2002) suggest that ammonia is a weak attractant, but in two of these, the release rate was only 0.65–0.70 mg/h. Fly responses to lures and trap type also depended on the site, suggesting tree size, spacing, and other factors affected how well the flies detected ammonia or the traps.

The required ammonia release rates for maximum captures of apple maggot were similar for yellow panels and red spheres. Although yellow panels were easier to use, red spheres captured more flies, and perhaps should be the trap of choice for monitoring programs. A monitoring system that incorporates red spheres and a sustained ammonia release of at least 1.4–4.7 mg/h may be needed to consistently detect low apple maggot populations in programs designed to prevent fly establishment in new areas.

We thank R Goughnour, T Coate, M Nash, S Jarman, and P Chapman for their field and laboratory assistance; B Wolfley, L Tanigoshi (Vancouver), and J Stark (Puyallup) of Washington State University for providing laboratory space; and G Fauth, D Dewey, S Donaldson, D Harkenrider (Area Manager), and the staff of the United States Department of Agriculture Forest Service, Columbia River Gorge National Scenic Area, for use of study sites. We also thank V Jones and M Klaus for critically reviewing the manuscript. This research was partially funded by the Washington Tree Fruit Research Commission.

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(Received: 2 June 2003; accepted: 6 October 2003)