Is the Concept of Short Retention Times for Aphid-Borne Nonpersistent Plant Viruses Sound?

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In an insect vector, if a virus neither multiplies nor circulates, exhibits no discernible latent period between acquisition and transmission, is lost during multiple probing behavior on plants, and is not retained after molting, it is defined as "nonpersistent." In aphids, the longest time interval between virus acquisition from an infected plant and transmission to an uninfected plant is the maximum retention time. For more than 50 yr it has been widely accepted that nonpersistent, aphid-transmitted viruses have very short maximum retention times. Retention times ranging from a few minutes to at most a few hours are reported in almost all literature in plant pathology and plant virology dealing with nonpersistent aphid transmission. The concept of retention time is central to understanding of plant viruses relative to spread, epidemiology, and transmission characteristics.

It is often assumed that nonpersistent aphid-borne viruses cannot be spread great distances because of short retention times. In general plant pathology and epidemiology this concept precludes consideration of long-distance transport and spread of nonpersistent viruses (2,4). Nevertheless, many winged aphid species (alates), including known virus vectors, are transported long distances by wind (6,7,14,15,17–19,22,25,27), many nonpersistent viruses are widely distributed, and unexpected epidemics and virus distributions remain an enigma (2,14–16,25,28). We suggest that many observations of short retention times may be artifacts, and that nonpersistent viruses may be retained throughout long-distance aphid dispersal and migration. It appears that reports of short retention times for nonpersistent viruses may have become incorrectly presumed because of the reliance of most investigators on confinement of insects in solid surface containers when measuring retention times, and the use of small numbers of insects.

Standard procedure for obtaining retention times. To date, almost all retention time experiments with aphid-borne nonpersistent viruses use the same basic design: After an acquisition period (usually preceded by aphid starvation to stimulate probing behavior) active insects are removed from source plants, confined in solid surface containers (usually petri dishes held at room temperature) for various time intervals, and then placed on assay plants. The time insects spend in solid surface containers is included in the retention time measurement. However, in nature, large numbers of alates fly from source plants, are caught in thermal updrafts or other wind currents (7,15,18), and can travel great distances (6,10,14,15,22,25,27). When aloft, aphids are often at lower temperatures than are found at crop levels, have no access to solid surfaces, and their dispersal occurs with or without active flight movements (17,18,28).

Solid surface containment. We suggest that confined aphids lose virus by attempting to test probe and salivating on solid surfaces. Heinze (13), using potato virus Y (PVY) potyvirus and cucumber mosaic virus (CMV) cucumovirus with *Myzus persicae* Sulzer, reported that aphids that had acquired PVY and CMV and were immobilized immediately by cold (−1 C) temperature, transmitted these viruses after 6 days and 40 hr, respectively. These aphids were denied any type of active behavior, including attempted probing. It is possible, however, that the cold treatment stabilized the virions or inactivated aphid salivary secretions which could otherwise destroy viral infectivity. Other evidence that solid surface confinement of active, viruliferous aphids affects subsequent virus transmission comes from Hashiba and Misawa (11,12) who worked with *M. persicae* and bean yellow mosaic (BYMV) potyvirus. They reported that aphids kept in glass containers at room temperature touched their rostrums to the glass in attempted test-probing behavior and thereon deposited saliva and BYMV particles. Although they found no inactivation of BYMV by aphid saliva (12) transmission efficiency of aphids that attempted probing and salivated on glass during a 10-min confinement was only half that recorded for nonprobing individuals (11).

In 1977 a maize dwarf mosaic virus (MDMV) potyvirus epidemic affecting parts of Canada and states of the United States of America bordering Canada, where MDMV is not endemic, forced us to consider various aspects of long-distance transport of aphids capable of vectoring this nonpersistent virus (1,4,5,28). In our laboratories we observed that both alates and apterae (wingless forms) of the greenbug, *Schizaphis graminum* Rondani, attempted to probe glass and plastic surfaces leaving behind salivary secretions (1). We prevented attempted probing of solid surfaces after allowing insects acquisition access to MDMV (strain A) by immobilizing them using either a 6 C cold treatment or by using argon (Ar) or nitrogen (N_2) gas as anaesthesia at 25 C (3). These treatments allowed direct comparison of immobilized alates and apterae, caused fewest behavioral side effects, and the 25 C temperature simulated temperatures found in low-level jet winds upon which aphids may have migrated in the 1977 MDMV epidemic (3–5,28). Carbon dioxide gas often used for insect anaesthesia was found unsuitable for these experiments because it causes undesirable behaviorial side effects and high mortality when used (3).

We then compared transmission efficiencies of immobilized and active alates (5,939 total insects used) and apterae (9,341 total insects used) throughout a 7-hr period (4). Regression analysis of transmission data throughout the 7-hr period showed clearly that preventing attempted probing behavior greatly increased MDMV transmission frequencies in immobilized insects (4). Also, the proportion of insects transmitting MDMV-A was significantly different, at 7 hr, using a binomial ratio comparison. Only 2.8% of all alates and apterae transmitted MDMV-A when allowed to attempt to probe solid surfaces, whereas 5.4% of alates and 5.5% of apterae transmitted virus when prevented from attempting probing by cold treatment at 6 C. At 25 C, using gaseous anaesthesia, 7.4% of alates and 7.8% of apterae prevented from attempted probing by N_2 immobilization transmitted MDMV-A, and 10.2% of alates and 8.8% of apterae transmitted virus after Ar immobilization. Because cold treatment at 6 C was less effective for increasing virus transmission than were gaseous treatments at 25 C, arguments of cold stabilization of virions

or effect of cold on salivary components which might reduce virus inoculativity appear unfounded (4). Our evidence confirmed and extended the reports of Heinze (13) and Hashiba and Misawa (11,12) who first implicated attempts to probe solid surfaces as a negative factor in determining retention times for nonpersistent viruses.

Because our work with MDMV-A required assaying 15,280 aphids on individual assay plants (4) it became impractical to repeat this particular experimental design for testing retention times longer than 7 hr with MDMV-A and with MDMV-B and other viruses. However, if we assumed that prolonged retention times were characteristic of populations of insects whose transmission frequencies were <5% it appeared reasonable to use five to 20 insects on each assay plant, and this logic led us to consider the effects of sample size relative to the probability of detecting virus transmission.

Small sample size. In all the retention time literature we reviewed, generally 10–50 aphids were assayed after each confinement interval. Rarely were 100 insects or more used for retention time experiments. The ability of aphids to transmit nonpersistent viruses decreases with time after acquisition, with or without denial of solid surface probing (2,4). Therefore, the proportion of insects transmitting after longer time intervals following acquisition, such as 20 hr, is considerably lower than after shorter time intervals. If, as in most published retention time literature, only 10–50 aphids are tested at each time interval, it is questionable whether these sample sizes were large enough to detect low transmission rates. One statistical approach to estimating necessary sample size involves independent Bernoulli trials and construction of a table of sample size versus probability of detection (21,24). Use of such a table (Table 1) illustrates the problem of transmission with a small sample size; for example, to be 99% certain of detecting a 3% transmission rate requires assay of 151 insects, and lower transmission rates require much larger sample sizes to be 95–99% certain of detection.

We used large numbers of aphids to measure retention times and assumed that a low percentage (<5%) of insects in any population would transmit after 7 hr (4). We used *M. persicae*, *S. graminum*, *Dactynotus ambrosiae* Thomas, *Rhopalosiphum maidis* Fitch, and *Macrosiphum euphorbiae* Thomas, to detect transmission of MDMV-A and MDMV-B at 18–70 hr depending on experimental conditions (4,28). Previously published retention times for MDMV ranged from 1 hr with 24 *M. persicae* to 6 hr with 10 *S. graminum* (23,26). Even when allowing attempted probing to occur, but using 575 *S. graminum*, we detected transmission at 21 hr after MDMV acquisition. However, denial of probing behavior in our experiments greatly increased our chances of detecting transmission when smaller sample sizes were used. In experiments with MDMV-F and *R. maidis*, and with *M. persicae* and soybean mosaic potyvirus, transmission at 24 hr was detected if we prevented probing behavior with cold treatment and used sample sizes as small as 25 insects (1). Maximum retention time experimentation with these nonpersistent viruses was discontinued when it was obvious that retention times in excess of 20 hr could be obtained by using adequate sample sizes, with or without denial of attempted solid surface probing behavior, and within the limits of aphid longevity. Historically, sampling small numbers of insects in retention time experiments undoubtedly contributed to a failure to detect a low frequency of virus transmission, and most probably resulted in reporting short retention times for many nonpersistent viruses.

TABLE 1. Sample size needed at various probabilities of detection when the frequency of transmission varies from 0.001 to 0.300

Transmission frequency (T)	Probability of detection (P)[a]			
	0.80	0.90	0.95	0.99
0.001	1,609[a]	2,301	2,994	4,602
0.003	536	766	997	1,532
0.005	321	459	598	919
0.010	160	229	298	458
0.030	53	76	98	151
0.050	31	45	58	90
0.100	15	22	28	44
0.300	5	6	8	13

[a]Based on the formula: $n \log (1 - T) = \log (1 - P)$; where n is the sample size required, T is the frequency of virus transmission of an aphid population, and P is the probability of detection (21,24).

Implications of longer retention times for nonpersistent viruses. There are innumerable records demonstrating mass, wind-assisted dispersal, and migrations of insects in many parts of the world, where various types of regularly occurring synoptic weather patterns are responsible for transport (6,10,16–18,25). However, weather patterns leading to formation of low-level jet winds on the North American Great Plains (5) are the most studied in relation to mass transport of aphids. Documented reports of presumed nonstop aphid transport on these low-level jet winds for distances of 360–1,600 km (17–19,22,27) are useful when calculating how long aphids remain aloft. At the 80 km/hr wind speeds common in low-level jet winds (2,28), a distance of 320 km is covered in 4 hr, and 1,600 km in 20 hr. We suggest that retention times for many nonpersistent aphid-borne viruses may, if measured, easily match these transport times. Long-distance transport of nonpersistent viruses must be a consideration when aphid transport occurs rapidly and as a nonstop event.

The low proportion but significant numbers of aphids that transmit at longer retention times facilitate long-distance virus transport. Aphid migrations and transport can occur en masse. There are reports of small aircraft encountering large "green clouds" of aphids on the North American Great Plains and one of our co-investigators was forced to land his aircraft when impacted aphids obscured his visibility (28). When massive numbers of alate migrate and a proportion of them are viruliferous, albeit small, there can be major epidemiological implications. Dickson (10) provided a classic example of the magnitude of an aphid migration when reporting that an estimated 23 billion alates passed over a one-mile portion of the Mojave Desert of California in a 3-hr period. If we assume that only 1% of this population is inoculative at takeoff and use the 3-hr half-life determined previously (4), there would be nearly 180 million inoculative vectors after 21 hr, easily enough to establish a plant virus epidemic in a localized area of susceptible plants. Because aphids indiscriminately attempt to probe glass, plastic, and plants, it is conceivable that long-distance viruliferous migrants can start epidemics, not colonize the plants they inoculate, and be long gone by the time symptoms appear (28).

Certainly, for transmission to occur two other criteria must be met: that the aphid is viruliferous on takeoff and that the aphid first lands on a virus-susceptible host plant rather than a nonhost. Little is known about precisely what aphids do just before takeoff. Cockbain et al (8) did note, however, that it was common for *M. persicae* and *Aphis fabae* to probe briefly just before takeoff. Cockbain and Heathcote (9) found that alates of *M. persicae* that developed on and flew from virus-infected sugar beets were about 16% efficient as vectors of the beet mosaic potyvirus, while *A. fabae* did not transmit under these conditions. These observations are rather limited in scope, but when coupled with the observation that natural transmission and epidemics occur, then aphid behavior must under certain environmental conditions be conducive to relatively high levels of inoculativity just before migration. Of course, since inoculativity is rapidly lost by feeding aphids (20) and since serial (plant-to-plant-to-plant) transmission rates of nonpersistent viruses are very low, then long-range migrants must not land and probe nonhosts before finding susceptible plants. Thus, aphid migrations where acquisition of nonpersistent viruses is followed by multiple landings and takeoffs would probably not result in long-range virus dispersal. Nonstop migration from source plants to susceptible plants is most likely the way nonpersistent viruses undergo long-distance dissemination.

In summary, we believe the concept of short retention times for nonpersistent aphid-borne viruses is invalid and should be challenged. As a concept, short retention times for nonpersistent

viruses should no longer constrain epidemiological consideration of long-distance virus spread. Most likely short retention times for nonpersistent viruses are laboratory artifacts caused by allowing inoculative, active aphids to attempt probing on solid surfaces during retention time experiments, and by use of small numbers of aphids assayed. For retention times to be meaningful, methodology should attempt to mimic natural conditions aphids encounter after virus acquisition and during migrations. If short retention times are methodology-induced artifacts then we suggest that the term nonpersistent be changed in favor of a more descriptive term like "probing-mediated persistence."

LITERATURE CITED

1. Berger, P. H. 1980. The retention of infectivity of maize dwarf mosaic virus in aphids (implications for long-distance dispersal). M.S. thesis. University of Minnesota, St. Paul. 70 pp.
2. Berger, P. H., and Ferriss, R. S. 1989. Mechanisms of arthropod transmission of plant viruses. Pages 40-84 in: The Spatial Component of Plant Epidemics. M. J. Jeger, ed. Prentice-Hall Publishing, Englewood Cliffs, NJ.
3. Berger, P. H., and Zeyen, R. J. 1987. Effects of sustained immobilization on aphids. Ann. Appl. Biol. 111:247-256.
4. Berger, P. H., Zeyen, R. J., and Groth, J. V. 1987. Aphid retention of maize dwarf mosaic virus (potyvirus): Epidemiological implications. Ann. Appl. Biol. 111:337-344.
5. Bonner, W. O. 1968. Climatology of the low level jet. Mon. Weather Rev. 96:833-850.
6. Close, R. C., and Tomlinson, A. I. 1975. Dispersal of the grain aphid *Macrosiphum miscanthi* from Australia to New Zealand. N. Z. Entomol. 6:62-65.
7. Coad, B. R. 1931. Insects captured by airplane are found at surprising heights. Pages 320-323 in: USDA Yearb. Agric.
8. Cockbain, A. J., Gibbs, A. J., and Heathcote, G. O. 1963. Some factors affecting the transmission of sugar beet mosaic and pea mosaic viruses by *Aphis fabae* and *Myzus persicae*. Ann. Appl. Biol. 52:133-143.
9. Cockbain, A. J., and Heathcote, G. D. 1964. Transmission of sugar beet viruses in relation to the feeding, probing and flight activity of alate aphids. Pages 521-524 in: Proc. Int. Congr. Entomol, 12th, London.
10. Dickson, R. C. 1959. Aphid dispersal over southern California deserts. Ann. Entomol. Soc. Am. 52:368-372.
11. Hashiba, T., and Misawa, T. 1969. Studies on the mechanism of aphid transmission of stylet-borne virus (III) on the adherence of the virus to the stylet. Tohoku J. Agric. Res. 20:159-171.
12. Hashiba, T., and Misawa, T. 1970. Studies on the mechanism of aphid transmission of stylet-borne virus (VI) effect of the saliva of the aphid. Tohoku J. Agric. Res. 21:73-87.
13. Heinze, K. 1959. Uber das verhalten unbestandiger phtopathogener viren bei der ubertragung durch blattlause. Phytopathol. Z. 36:131-145.
14. Hodson, A. C., and Cook, E. F. 1960. Long-range aerial transport of the harlequin bug and the green bug into Minnesota. J. Econ. Entomol. 53:604-608.
15. Holzapfel, E. P., Tsuda, E. M., and Harrell, J. C. 1970. Trapping of air-borne insects in the antarctic area (part 3). Pac. Insects 12:133-156.
16. Irwin, M. E., and Thresh, J. M. 1988. Long-range aerial dispersal of cereal aphids as virus vectors in North America. Philos. Trans. R. Soc. London B 321:421-446.
17. Johnson, C. G. 1967. International dispersal of insects and insect-borne viruses. Neth. J. Plant Pathol. 73 (Supp. 1):21-43.
18. Johnson, C. G. 1969. Migration and Dispersal of Insects by Flight. Barnes and Noble, Inc., New York. 763 pp.
19. Medler, J. T., and Smith, P. W. 1960. Greenbug dispersal and distribution of barley yellow dwarf virus in Wisconsin. J. Econ. Entomol. 53:473-474.
20. Nault, L. R., and Bradley, R. H. E. 1969. Acquisition of maize dwarf mosaic virus by the greenbug, *Schizaphis graminum*. Ann. Entomol. Soc. Am. 62:403-406.
21. Parzen, E. 1960. Modern Probability Theory and Its Application. John Wiley & Sons, Inc., New York. 464 pp.
22. Peterson, A. G., Bates, J. O., and Saini, R. S. 1968-1969. Spring dispersal of some leafhoppers and aphids. J. Minn. Acad. Sci. 35:98-102.
23. Shaunak, K. K., and Pitre, H. N. 1973. Comparative transmission of maize dwarf mosaic virus by *Aphis fabae*, *Aphis gossypii*, and *Schizaphis graminium*. Plant Dis. Rep. 54:533-536.
24. Swallow, W. H. 1985. Group testing for estimating infection rates and probabilities of disease transmission. Phytopathology 75:882-889.
25. Thresh, J. M. 1983. The long-range dispersal of plant viruses by arthropod vectors. Philos. Trans. R. Soc. London B 302:497-528.
26. Tosic, M., and Sutic, D. 1968. Medusobni odnos virusa mozaika kukuruza neki njegovih vektora. Acta Biol. Iugosl. Ser. B 5:73-78.
27. Wadley, F. M. 1931. Ecology of *Toxoptera graminum* especially as to factors affecting importance in the northern United States. Ann. Entomol. Soc. Am. 24:325-395.
28. Zeyen, R. J., Stromberg, E. L., and Kuehnast, E. L. 1987. Long-range aphid transport hypothesis for maize dwarf mosaic virus: History and distribution in Minnesota, USA. Ann. Appl. Biol 111:325-336.