April 2014

Desirable Host Plant Qualities in Wild Rice (*Zizania Palustris*) for Infestation by the Rice Worm *Apamea Apamiformis* (Lepidoptera: Noctuidae)

Nathan B. Dahlberg
University of Minnesota

John Pastor
University of Minnesota

Follow this and additional works at: https://scholar.valpo.edu/tgle

 Part of the Entomology Commons

Desirable Host Plant Qualities in Wild Rice (*Zizania palustris*) for Infestation by the Rice Worm *Apamea apamiformis* (Lepidoptera: Noctuidae)

Nathan B. Dahlberg[1] and John Pastor[2]*

Abstract

The rate at which an insect infests hosts by ovipositing and/or subsequent growth of larvae often depends on specific desirable host plant qualities. In this study, we measured the infestation rate of wild rice, *Zizania palustris,* by the wild rice worm, *Apamea apamiformis*, D. F. Hardwick (Lepidoptera: Noctuidae) and compared it to sediment nitrogen availability, plant biomass, plant density, litter accumulation, and seed carbohydrate and nitrogen concentration. Plant density and litter accumulation had no effect on infestation rates. Infestation rate increased with plant biomass and sediment nitrogen availability. The correlation between infestation rate and sediment nitrogen availability seems to reflect the fact that high nitrogen availability produces larger plants rather than more nutritious seeds as the infestation rate was not correlated with seed glucose content and surprisingly decreased with concentration of nitrogen in seeds. Infestation rate was not related to any other measured quantities. Therefore, *Apamea* appear to infest larger, rapidly growing host plants which are made possible by high sediment nitrogen availability.

The rate at which an herbaceous insect infests its host by ovipositing and/or subsequent growth of larvae is thought to be directly related to desirable host qualities (Awmack and Leather 2002, Wiesenborn and Pratt 2008). Host selection of their food plants is thought to be essential to the fecundity of many insects (Awmack and Leather 2002). Factors such as host plant size (Heisswolf et al. 2005), leaf tenderness (Takagi and Miyashita 2008), host plant density (Rauscher 1983, Courtney and Forsberg 1988, Chew and Courtney 1991), and nitrogen content of plant tissues (White 1993, Awmack and Leather 2002, Wiessenborg and Pratt 2008), among many other qualities, can be some of the host qualities desired by many species of Lepidoptera.

Apamea apamiformis D. F. Hardwick, a member of the Noctuidae, the largest family in Lepidoptera, is the most important pest of wild rice (*Zizania palustris*) in the Upper Great Lakes region (Aiken et al. 1988). Despite its importance to this commercially valuable aquatic grain, little is known about the ecology of *A. apamiformis,* commonly but incorrectly known as the "rice worm" even though it is the larva of a lepidopteran. To our knowledge, there have been only two studies on the development and emergence of *A. apamiformis* (MacKay and Rockburne 1958, Melvin 1966). MacKay and Rockburne (1958) were the first to publish taxonomic characteristics of the larva and classified it into the genus *Apamea*. They were also the first to describe its life cycle. Eggs of *A. apamiformis*

[1]Department of Biology University of Minnesota Duluth, 1035 Kirby Dr. Duluth Minnesota, 55811, USA. (e-mail: dahlb093@d.umn.edu).
[2]Department of Biology, University of Minnesota Duluth, 1035 Kirby Dr, Duluth, Minnesota 55811, USA, Telephone: (218) 726-7001 Fax: (218)726-8142. (e-mail: jpastor@d.umn.edu).
*Corresponding author.

are laid in July inside the floret of wild rice flowers when the flowers are open. If no pollination occurs, the flowers will remain open for 2-3 days, giving adult females time to deposit hundreds of eggs (Mackay and Rockburne 1958). As larvae develop, they typically feed on different plant organs. Early instars feed on seed heads, leaving a characteristic hole in the seed coat, while later instars feed on heads as well as stalks. It is not known whether larvae move among different inflorescences, but given that the *Zizania* seed heads are usually approximately 20-30cm apart, movement of larvae between plants seems unlikely. This assumption requires additional research. As development progresses and the growing season ends, late instars seek an alternative habitat, thought to be wild rice litter on the shore (Melvin 1966). There, they bore into rice stalks or in the ground (if available) and pupate and complete their life cycle in the spring, emerging in their adult form. The larvae that manage to reach the shore are thought to be the only larvae that survive (Melvin 1966).

Nitrogen limits wild rice vegetative and seed production (Walker et al. 2010, Sims et al. 2012a, b) and so may play a role in regulating both *Zizania* and *Apamea* populations. Wild rice populations undergo production and density oscillations on an approximately 4 year cycle, with productive years being followed by a crash and then a slow recovery over the next 2 or 3 years (Jenks 1899, Moyle 1944, Archibold and Weichel 1985, Aiken et al. 1988). The oscillations of wild rice are due to delays in nitrogen supply caused by microbial immobilization of nitrogen into fresh decaying wild rice litter (Pastor and Walker 2006, Walker et al. 2010). Eventually, nitrogen is released in the later stages of decomposition, allowing the wild rice populations to recover. A large amount of litter, produced by productive plant growth during the previous year, depresses sediment nitrogen availability, seed biomass, and seed nitrogen content the following year (Walker et al. 2006, 2010). *Apamea* populations also oscillate on a 4-year cycle (Melvin 1966), but Aiken et al. (1988) state that it is not clear whether the cycles of *A. apamiformis* populations are the cause or effect of cycles in wild rice populations. However, since host plant density, sediment nitrogen availability, and seed production and nitrogen content all vary during a wild rice population cycle (Walker et al. 2010) and they may each affect food supply and food quality to ovipositing females, it is possible that these plant attributes drive *A. apamiformis* population cycles by altering host preference and performance. The roles of plant density, litter production, sediment nitrogen availability, and seed nitrogen content on preference and performance of *A. apamiformis* need to be sorted out by manipulative experiments.

To understand the complicated relationships between nitrogen supply, wild rice productivity and density, and infestation rates by *A. apamiformis*, we must first understand the factors by which *A. apamiformis* infests host plants, including plant density as well as nitrogen supply rates from the sediments and nitrogen concentrations in host plant tissues. In this study, we hypothesized that the number of seeds infested by *A. apamiformis* larva would vary with host plant density, litter amount, soil nitrogen availability and nitrogen concentrations in the seeds.

Methods

We took advantage of a larger study on productivity, nitrogen cycling, and population oscillations in wild rice to investigate factors controlling infestation by *A. apamiformis*. (see Walker et al. 2010 for details of the experimental design, but they are briefly reported here). Wild rice plants were grown at The University of Minnesota Duluth Research and Field Station in Duluth, Minnesota. Fifty four, 400 liter tanks were placed in the ground and filled with 10 cm of sediment overlying 10cm of sand. The sediment was collected from a lake on the Fond du Lac Reservation in Carlton County in northern Minnesota. Water was added to the tanks immediately after the sand and sediment layers

were placed in the tanks. The tanks were connected to a 20 L plastic bucket by a PVC pipe whose opening was 23 cm above the sediment surface. The water was refilled weekly to the height of the PVC pipe. This allowed us to keep the water level relatively constant and analyze any water draining into the bucket.

In the study of Walker et al. (2010), tanks were given two different treatments in a crossed factorial design. These treatments were either thinned (to 30 plants total) or unthinned, and either litter removed or litter retained. While these treatments were established to test hypotheses about the causes of wild rice population cycles (Walker et al. 2010), they can also help us understand factors influencing *A. apamiformis* infestation. Comparing thinned tanks to unthinned tanks allows us to determine if plant density is a factor determining *A. apamiformis* ovipositing and larval survival rates, as suggested by studies on other lepidopteran and other insect species (Rauscher 1983, Courtney and Forsberg 1988, Chew and Courtney 1991). Manipulation of the litter alters sediment nitrogen availability and consequently plant growth and seed nitrogen content (Walker et al. 2006, 2010), so the direct effects of litter accumulation as well as its influence on sediment and plant nitrogen and how they indirectly influence *A. apamiformis* can also be tested. These two treatments were applied in a random factorial design across 54 tanks, yielding four combinations.

During the growing season, we selected a representative sample of six *Zizania* plants from each tank for detailed measurements of biomass and its allocation amongst roots, shoots, seeds and nitrogen content. The seeds of these plants were counted and classified into full, empty, and *Apamea* infested, so that larval feeding activity can be calculated in relation to nitrogen content of the plants and nitrogen availability in the tanks. These six plants were harvested after they senesced in October and were dried to determine moisture contents. All aboveground biomass in the tanks was also collected and weighed, and their dry biomass determined by multiplying the wet weight by the dry: wet biomass ratio of the six sampled plants.

Plants were counted in early September. Plant density was calculated by dividing the number of plants per tank by the area of the tank (0.54 m²). Plant biomass was measured in late autumn after *Zizania* had completely senesced. The total dry biomass in each tank was divided by the number of plants per tank. This value gave us the mean plant weight per tank.

Wild rice seeds were collected beginning in August and ending in September of 2011. We picked seeds every other day until every seed was harvested from the 6 sample plants from each tank. Seeds were placed in a drying oven at 60° C for at least 24 hours to inhibit fungal growth and kill remaining larvae that may have been collected in the envelopes used to collect seeds. After the seeds were dry, total seed number, viable seed number, number of seeds with larvae feeding holes, viable seed weight, peduncle number, and peduncle weight were measured on each plant. Viable seed number and weight correspond with biomass and productivity of wild rice (Sims et al. 2012b). The peduncle number and total seed number were compared to determine the error in collecting seeds. Usually, 2-3 seeds were not collected because they ripened and fell into the tanks before they had been sampled. Mean viable seed weight was calculated by multiplying the ratio of viable seeds to total seeds by the number of seeds counted on the peduncle. This number was then multiplied by the average viable seed weight to estimate total viable seed weight per plant.

After all seeds were counted, the seeds from the six individual plants from each tank were combined into 54 composite samples and ground in a seed sample grinder. After seeds were ground, 5 µg of sample was saved for nitrogen and glucose analysis. Nitrogen content was analyzed using a Thermo Scientific FLASH EA™ 1112 Elemental Analyzer. Glucose was measured to determine the effects of carbohydrates on *Apamea* infestation. We extracted starches from 10

randomly selected infested tanks and 10 randomly selected uninfested tanks by hot water starch extraction. After seeds were ground, they were weighted and placed in a 75mL volumetric tube. The tubes were filled with deionized water and heated to 103° C for three hours. We then performed a phenol-sulfuric acid assay to test for sugars (Dubios et al. 1956).

Sediment N availabilities were measured with three mixed bed resin ion exchange bags (Binkley 1984) placed in the sediment in each tank in spring and harvested at the end of the 2011 growing season. The resins (Fisher Rexyn 300) were then extracted using 1N KCl, which was then analyzed for NH_4 and NO_3, using Lachat Autoanalyzer methods 10-107-06-1-J and 10-107-04-1-A, respectively.

Seed infestation rate was calculated as the proportion of the total number of seeds from the six sampled plants infested with feeding holes. Plant infestation rate was calculated as the proportion of the six sampled plants per tank infested with at least one *Apamea* larva. These two measurements were used to analyze two different levels of infestation.

The main (and direct) effects of plant density (via thinning) and litter amount (via litter removal) on seed and plant infestation rates were tested using a randomized complete block, 2-factor ANOVA with interaction terms after normalizing the data with an arc sine square root transformation and ensuring equal variances. Plant and seed infestation rates were also regressed against seed nitrogen and carbohydrate concentrations, resin bag nitrogen availability (µg/g resin), total stem density per tank, average plant biomass, seed biomass per plant, and average height of the six sample plants to determine which of these factors potentially determined preferences of the female adult moths for host plants.

Results

Neither seed infestation rate (proportion of the number of seeds on a plant which were infested by *Apamea*) nor plant infestation rate (proportion of plants in a population infested with at least one *Apamea* larva) were directly affected by either thinning the host plant density ($F = 2.95$, df = 1, $P = 0.09$ for seed infestation rate and $F = 3.27$, df = 1, $P = 0.08$ for plant infestation rate) or by litter removal ($F = 0.812$, df = 1, $P = 0.37$ for seed infestation rate and $F = 0.332$, df = 1, $P = 0.57$ for plant infestation rate). There were no statistically significant interactions between host plant density and litter removal on either seed or plant infestation rates.

However, seed infestation rate increased as both plant and seed biomass increased. Seed infestation was more strongly correlated with plant biomass ($P = 0.007$; $r^2 = 0.14$) than it was with seed biomass ($P = 0.030$; $r^2 = 0.09$).

Plant infestation rate increased with seed ($P = 9.8E-5$; $r^2 = 0.19$) and plant biomass ($P = 5.8E-6$; $r^2 = 0.33$) even more strongly than seed infestation. *A. apamiformis* may select how many plants to infest per tank based on plant and seed biomass and/or the survival rate of their larvae is enhanced on larger plants (Fig. 1). However, the correlation between plant biomass and seed and plant infestation rates are not solely functions of plant height because mean plant height was not correlated with plant infestation rate nor with seed infestation rate ($P = 0.10$ and $P = 0.28$; respectively). These results suggest that plant height *per se* does not play a role in determining infestation rate of *A. apamiformis*, but rather that *A. apamiformis* infestation rate is greater on larger and more robust plants.

The greater infestation rate on larger plants may be an indirect result of sediment nitrogen availability because the proportion of infested seeds per plant increased with sediment nitrogen availability ($P = 0.047$, $r^2 = 0.37$, Fig. 2a).

There was no significant difference in glucose concentrations in seeds between infested and uninfested tanks ($P = 0.96$). In contrast, seed infestation
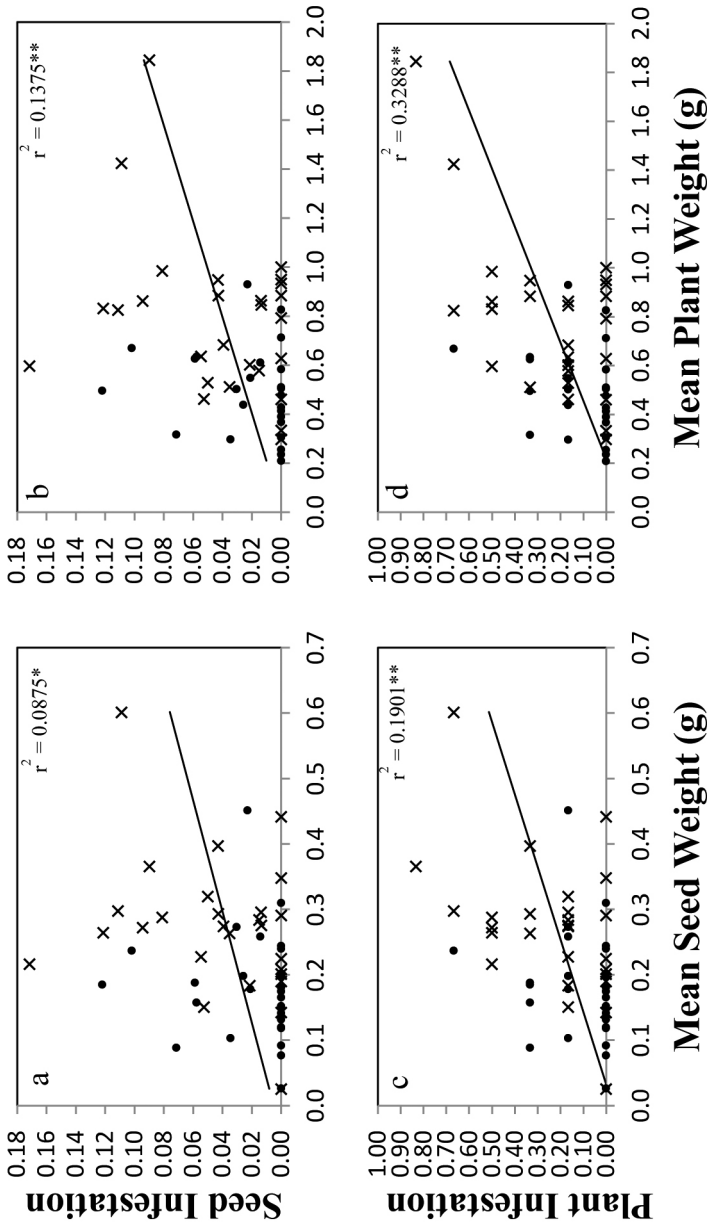
Figure 1. The comparison of seed and plant biomass to infestation rate. Both seed and plant infestation rate increases as seed weight and plant weight increases. Fig. 1a and Fig. 1b represent seed and plant biomass compared to seed infestation, and Fig. 1c and Fig. 1d represent seed and plant biomass compared to plant infestation. Plant infestation rate is more significant than seed infestation. Filled circles represent non-thinned tanks and Xs represent thinned tanks. r^2 values are shown (* and ** represent $P < 0.05$ and $P < 0.01$ respectively).
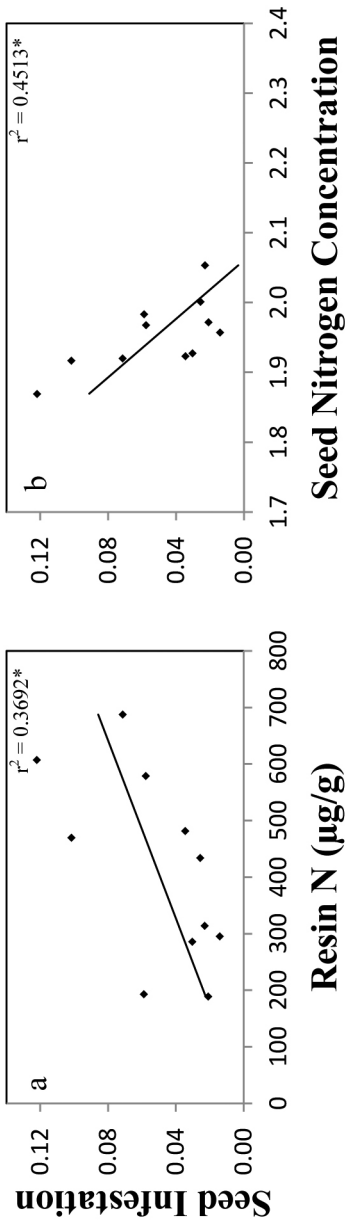
Figure 2. Resin bag nitrogen and percent seed nitrogen correlated to seed infestation rate. Resin bag nitrogen and seed nitrogen show an opposite relationship. As resin nitrogen increases, seed infestation increases (Fig. 2a), but infestation rate decreases with increasing seed nitrogen concentration (Fig. 2b). Fig. 2a, $P = 0.047$; Fig. 2b $P = 0.024$.

rate declines with the concentration of nitrogen in seeds ($P = 0.024$, $r^2 = 0.45$, Fig. 2b). Surprisingly, seed nitrogen concentration was not correlated with sediment nitrogen availability ($P = 0.671$, $r^2 = 0.005$).

Discussion

A. apamiformis does not appear to preferentially infest taller plants, plants with high seed glucose concentrations, or plants in dense populations. Instead, *A. apamiformis* infestation rate increased with individual plant biomass (Fig. 1). Since wild rice is an annual plant, the largest plants were the ones that grew fastest that growing season. Ovipositing females may select large plants with rapid growth rate; alternatively (or in addition) plants with rapid growth rate may provide larvae with abundant food. Either or both of these processes could increase both plant and seed infestation rate. Plant infestation rate was more strongly related to plant biomass than it was to seed biomass. Seed infestation rate was less strongly related to individual plant biomass than plant infestation rate. This suggests a selective pressure for adult moths to lay eggs on large, rapidly growing plants, but the effect of rapid growth on seed infestation within a plant was not as strong. These results are consistent with the plant vigor hypothesis (Price 1991, 2003; Craig et al. 1986, 1989; Heisswolf et al. 2005) which proposes that infestation rate increases with plant growth rate

A. apamiformis infestation also increased with sediment nitrogen availability but this may be because wild rice productivity also increases with sediment nitrogen availability (Walker et al. 2010, Sims et al. 2012a). Sims et al. (2012b) found that *A. apamiformis* infestation rates of plants increased with added fertilizer nitrogen, which is in support of our findings of a correlation fo sediment nitrogen availability and infestation rates. The correlation between infestation rate and nitrogen availability may therefore reflect the fact that high nitrogen availability produces larger plants. Therefore, *A. apamiformis* appear to infest larger, rapidly growing host plants which are made possible by high sediment nitrogen availability.

Surprisingly, even though it increased with increasing sediment N availability seed infestation rate decreased with increasing seed N concentration (Fig. 2). Therefore, infestation rate did not increase with sediment nitrogen availability because seeds were more nutritious. Neither did infestation rates increase with glucose concentrations. Surprisingly, seed nitrogen concentration and sediment nitrogen availability were not correlated with one another, suggesting that these two factors act independently of one another on infestation rates. These data suggest that there may be some type of nitrogenous secondary metabolite in *Zizania* seeds that *A. apamiformis* is avoiding and which may be under control of other factors independent of sediment nitrogen availability. Although alkaloids have been found in several members of the Poaceae (Indu and Onyibe, 2011), the family which includes wild rice, the presence of alkaloids have not yet been detected in wild rice or any other species of the same genus.

Sunlight is thought to be one of the important factors for producing secondary metabolites (Jansen and Stamp, 1996) which may deter adult moths from laying eggs on plants in full sunlight. Full sunlight and high nitrogen availability together can lead to high energy stores and surplus N supplies, both of which together may allow the plant to produce more alkaloids, thus reducing host quality. Sims et al. (2012b) found that *A. apamiformis* infection rates on wild rice increased when plants grown with added nitrogen were also shaded, which lends support to this hypothesized mechanism. Experiments should be done manipulating both nitrogen and light availabilities and measuring seed nitrogen content, alkaloid concentrations, as well as *A. apamiformis* infestation rates, fecundity, larval growth, and consumption rates (Awmack and Leather 2002).

Our experiment could not distinguish whether the high infestation rates on larger plants were due to host selection by ovipositing females or by greater

larval survival and development or both. Distinguishing the effects of host selection and larval survival on infestation rates would require observations on both ovipositing adult female moths as well as larval survival and growth rates on *Zizania* grown under different nitrogen and light availabilities (Craig et al. 1989).

In summary, *A. apamiformis* infestation rate increases in wild rice plants with high seed and plant biomass growing in sediments of high nitrogen availability but decreases in plants with seeds that have high N concentrations, which may be due to the presence of alkaloids in the seeds. Seed carbohydrate content, plant density, and plant height do not seem to affect how larvae infest plants. Our data suggests that desirable host plant qualities include high biomass and low seed (or at least not high) N concentrations.

Acknowledgments

Literature Cited

Aiken S. G., P. F. Lee, D. Punter, and J. M. Stewart. 1988. Wild Rice in Canada. Toronto: NC Press Ltd.

Archibold, O. W., and B. J. Weichel. 1985. Variation in wild rice (*Zizania palustris*) stands across northern Saskatchewan. Canadian Journal of Botany 64: 1204-1211.

Awmack, C. S., and S. R Leather. 2002. Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology 47: 817-844.

Binkley, D. 1984. Ion exchange resin bags: factors affecting estimates of N availability. *Soil* Science Society of America Journal 48: 1181–1184.

Chew, F. S., and S. P. Courtney. 1991. Plant apparency and evolutionary escape from insect herbivory. American Naturalist 138: 729-750.

Courtney, S. P., and J. Forsberg. 1988. Host use by two pierid butterflies varies with host density. Functional Ecology 2: 67-75.

Craig, T. P., P. W Price., and J. K. Itami. 1986. Resource regulation by a stem-galling sawfly on the arroyo willow. Ecology 67: 419-425.

Craig, T. P., J. K. Itami, and P. W. Price. 1989. A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. Ecology 70: 1691-1699.

Dubois, M., K. A. Giles, J. K. Hamilton, J. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350-356.

Heisswolf A., E. Obermaier, and H. J. Poethke. 2005. Selection of large host plants for oviposition by a monophagous leaf beetle: nutritional quality or enemy-free space? Ecological Entomology 30: 299-306

Indu, M., and H. Onyibe. 2011. Nature of ergastic substances in some Poaceae seeds. African Journal of Biotechnology 10: 9800-9803.

Jansen, M. P. T., and N. E. Stamp. 1996. Effects of light availability on host plant chemistry and the consequences for behavior and growth of an insect herbivore. Entomological Experiment and Applications 82: 319-333.

Jenks, A. E. 1899. The wild rice gatherers of the Upper Great Lakes. Ph.D. dissertation, Department of Anthropology, University of Wisconsin, Madison.

MacKay, M. R., and E. W. Rockburn. 1958. Notes on life-history and larval description of *Apamea apamiformis* (Guenée), a pest of wild rice (Lepidoptera: Noctuidae). Canadian Entomologist 90: 579-582.

Melvin, J. C. E. 1966. Observations on insects attacking wild rice in Manitoba. Entomological Society of Manitoba 22: 6-11.

Moyle, J. B. 1944. Wild rice in Minnesota. Journal of Wildlife Management, 8: 177-184.

Pastor, J., and R. D. Walker. 2006. Delays in nutrient cycling and plant population oscillations. Oikos 112: 698-705.

Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. Oikos, 62: 244-251.

Price, P. W. 2003. Macroevolutionary theory on macroecological patterns. Cambridge University Press.

Rausher, M. D. 1983. Alternation of oviposition behavior by *Battus philenor* butterflies in response to variation in host plant density. Ecology 64: 1028-1034.

Sims, L., J. Pastor, T. D. Lee, and B. W. Dewey. 2012a. Nitrogen, phosphorus and light effects on growth and allocation of biomass and nutrients in wild rice. Oecologia 170: 65-76.

Sims, L., J. Pastor, T. D. Lee, and B. W. Dewey. 2012b. Nitrogen phosphorus and light effects on reproduction and fitness of wild rice. Botany 90: 876-883.

Takagi, S. and T. Miyashita. 2008. Host plant quality influences diapause induction of *Byasa alcinous*. Annals of the Entomological Society of America 101: 392-396.

Walker, R. D., J. Pastor, and B. Dewey. 2006. Effects of wild rice (*Zizania palustris* L.) straw on biomass and seed production in northern Minnesota. Canadian Journal of Botany 84: 1019-1024.

Walker, R. D., J. Pastor, and B. W. Dewey. 2010. Litter quantity and nitrogen immobilization cause oscillations in productivity of wild rice (*Zizania palustris* L.) in Northern Minnesota. Ecosystems 13: 485-98.

White, T. C. R. 1993. The inadequate environment: nitrogen and the abundance of animals. Springer-Verlag, Berlin.

Wiesenborn, W. D., and G. F. Pratt. 2008. Selection of *Atriplex lentiformis* host plants by *Hesperopsis gracielae* (Lepidoptera: Hesperiidea). Florida Entomologist 91: 192-198.