Role of the insect *Nysius niger*, and flixweed, *Descurainia sophia*, in infection of Saskatchewan mustard crops with a yeast, *Nematospora sinecauda*

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The coincidence of the two outbreaks of the yeast *Nematospora sinecauda* in the seed of mustard crops since 1981 with the two peaks of abundance of *Nysius niger* [formerly known as *N. ericae*] suggests that this insect is of major importance in transmitting the pathogen to the crop. The yeast was shown not to overwinter in *N. niger*, as only the egg stage of this insect survived the winter in southern Saskatchewan. No evidence of adult *N. niger* migrating into southern Saskatchewan from warmer southern latitudes was obtained, indicating that the yeast is not reintroduced in the spring by immigrant insects. Flixweed [*Descurainia sophia*], a common cruciferous weed on the prairies and the main early season host of *N. niger*, was also found to be a host for *N. sinecauda*. Laboratory experiments demonstrated that adults of *N. niger* could acquire *N. sinecauda* inoculum by feeding on naturally infected ripe flixweed seeds collected from the field. Field observations suggested that nymphs also could acquire the inoculum by this same route. *N. sinecauda* was found to survive the prairie winter in ripe infected seeds retained within the pods of mature flixweed plants. Such seeds would provide a source of inoculum for the vector insect in the spring. In conclusion, it appears that both *N. niger* and flixweed play a part in the infection of western Canadian mustard crops with *N. sinecauda*.

Can. Plant Dis. Surv. 71:1, 37-41, 1991.

La coincidence des deux épisodes d'infection des graines de moutarde par la levure *Nematospora sinecauda* depuis 1981 avec les deux pics d'abondance de *Nysius niger* [anciennement connu sous le nom de *N. ericae*], donne à penser que cet insecte est d'une grande importance dans la transmission de l'agent pathogène à la culture. Il a été prouvé que la levure n'hivernait pas dans *N. niger*, car seul le stade oeuf de cet insecte survit à l'hiver dans le sud de la Saskatchewan. On n'a aucune preuve voulant que des adultes de *N. niger* migrent dans le sud de la Saskatchewan à partir de latitudes plus chaudes situées plus au sud, ce qui renforce l'hypothèse voulant que la levure ne soit pas réintroduite au printemps par des insectes immigrants. Le sisymbre sophia [*Descurainia sophia*], mauvaise herbe crucifère commune dans les Prairies, principal hôte de *N. niger* en début de saison, s'avère également être un hôte de *N. sinecauda*. Des expériences en laboratoire ont révélé que des adultes de *N. niger* pouvaient acquérir de l'inoculum de *N. sinecauda* en se nourrissant de graines mûres de sisymbre naturellement infectées et récoltées dans le champ. Des observations en plein champ donnent à penser que les nymphes pourraient également acquérir l'inoculum par la même voie. On a constaté que *N. sinecauda* survivait à l'hiver des Prairies dans des graines mûres infectées contenues dans les gousses de plants adultes de sisymbre. Ces graines fourniraient une source d'inoculum à l'insecte vecteur au printemps. En conclusion, il semble que *N. niger* et le sisymbre jouent un rôle dans l'infection par *N. sinecauda* des cultures de moutarde dans l'ouest du Canada.

Introduction

In 1979, a yeast identified as *Nematospora coryli* Peglion was found infecting many commercial seedlots of mustard [*Brassica juncea* (L.) Coss.] grown in the Canadian Prairie Provinces, northern Montana and northern North Dakota (J.S. Hemingway, Colman Foods, Norwich, England 1980, unpub. report to the Mustard Association). Its presence resulted in undesirably high plate counts of microorganisms in condiment flours ground from such seed. Holley *et al.* (1984) reported that the yeast infecting mustard seed is a new and previously undescribed species of *Nematospora*, *N. sinecauda* Holley, Allan-Wojtas & Phipps-Todd, rather than *N. coryli*, which it closely resembles. *N. sinecauda* has also been detected in white mustard [*Sinapis alba* L.] (J.S. Hemingway 1980 unpub. report) and in canola [*Brassica napus* L.] (Burgess and McKenzie, unpublished data).

Burgess *et al.* (1983) identified a hemipterous insect, then commonly known as *Nysius ericae* (Schilling) or the false chinch bug, but now correctly named *Nysius niger* Baker (Ashlock 1977), as a vector of this yeast, but they did not discover how the yeast overwintered. Burgess, Verma and McKenzie (1982, unpublished report to the Mustard Association) determined that the yeast did not appear to overwinter in the soil, nor did sowing infected seed give rise to infected plants. Holley and Jones (1985) added support to the latter finding when they reported that 99.9% of the *Nematospora* originally present in oriental mustard seed was killed within 24 hr during germination of the seed between moist filter papers.

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Accepted for publication October 15, 1990.

The present paper reports further investigations of the role of *N. niger* in infecting commercial mustard crops with *N. sinecauda*, and of where the yeast inoculum overwinters. The studies were carried out in southern Saskatchewan, which constitutes the central portion of the western Canadian mustard growing region.

Materials and methods

N. niger nymphs and adults were collected by sweeping flixweed [*Descurainia sophia* (L.) Webb] with a heavy cloth insect net, or by collecting the insects directly from plants or the soil surface with an aspirator. Specimens brought into the laboratory for study or plating were transported in an insulated chest chilled with ice or freezer packs. Insects to be examined for the presence of *N. sinecauda* were frozen and stored at −10 to −12°C; then surface sterilized, crushed, and plated on Difco Standard Methods Agar, as outlined by Burgess *et al.* (1983). Similar procedures were used to detect yeast in flixweed seeds and seedlings.

N. niger populations were estimated by sweeping semi-permanent patches of flixweed. Because *N. niger* often congregated in a small area of a flixweed patch, while being absent or present in low numbers elsewhere in the patch, flixweed patches were not swept with a specific number of strokes. Rather, they were swept extensively to determine if the insects were apparently absent, present in low to moderate numbers, or abundant. In the abundant category, hundreds of insects would be obtained in the sweeps from a patch, usually from one or more dense aggregations of *N. niger.*

To check for the presence of overwintering *N. niger*, 0.25 m^2 samples of leaf litter and turf to a depth of 6 cm were taken in the autumn and winter near sites where *N. niger* was earlier present. This material was brought into the laboratory, warmed to 21-24°C, and hand sorted.

To help determine the developmental stage in which *N. niger* overwinters, in autumn 1984 an emergence cage 13.5 cm high and 0.5 m^2 in area, and additional protective mustard and wheat straw were placed over each of two aggregations of *N. niger* nymphs and adults in a mustard stubble field with unharvested swaths. Each cage enclosed insects on and under a swath, as well as some on the ground in the adjacent stubble. The straw, stubble and soil within the cages were examined carefully for surviving insects the following spring.

Ripened flixweed seeds were collected by sweeping mature plants with a heavy cloth insect net, or by collecting mature pod bearing stems and rubbing the seed out by hand. Seed was then cleaned with a series of fine metal sieves.

Feeding trials on ripened flixweed seed were carried out in the laboratory with field-caught or laboratory-reared (Burgess and Weegar 1986) *N. niger.* These trials were conducted in 13 × 13 × 9 cm clear polystyrene boxes lined on the bottom with paper towelling, with a 5 cm screened port in the lid partly covered with moist dental cotton to provide adequate humidity. In a trial, a number of adults or nymphs were introduced into a polystyrene box containing flixweed seeds on the towelling, and the insects were observed for feeding activity. Similar feeding tests were carried out with ripe *B. napus* and *B. juncea* seeds.

To detect the acquisition of yeast inoculum by *N. niger* feeding on ripened infected flixweed seeds, laboratory reared *N. niger* with no previous contact with *N. sinecauda* were used. The yeast-free status of the laboratory colony supplying the test insects was checked prior to each experiment by plating 10 or 12 insects chosen randomly from the colony. The acquisition trials were conducted in 6 × 2 cm disposable plastic Petri dishes with a filter paper liner on the bottom, and a 9 mm stoppered port on one side for introduction of insects and seeds. Moist dental cotton was again used to provide adequate humidity. In a trial, 50 *N. niger* adults or nymphs were introduced into a Petri dish containing infected flixweed seeds on the filter paper, except that only 24 nymphs were used in one test. The test insects were left in the Petri dish with access to the infected seeds for 24 to 52 hr, then removed and plated to see if they had acquired yeast inoculum by feeding.

To detect the transmission of yeast inoculum to *B. juncea* by *N. niger* adults that had fed on infected flixweed seed, a developing green pod of *B. juncea* was placed on a paper shelf within the Petri dish for the last 24 hr of a 48 hr inoculum acquisition experiment. The shelf kept the infected seed and the *B. juncea* pod physically separated, but allowed the insects ready access to both. Both the insects and the seeds of the test pod were plated for yeast at the end of the experiment. The pod used in this experiment was obtained from a greenhouse-grown yeast-free plot of *B. juncea*, with the pods immediately above and below it on the plant being plated as controls.

To simulate the overwintering of yeast infected flixweed seeds on the ground, which were otherwise difficult to separate from soil and debris, a sample of infected seed was placed on a soft cloth stretched across a small wooden frame. The frame and seed were placed on the ground in a fencerow in early November, and retrieved the following spring for plating. The stretched cloth held the seed just above the soil surface, and a metal screen covering protected the seed from rodent and insect damage.

Results and discussion

Relation of *Nematospora* outbreaks in mustard crops to *N. niger* abundance

Observations in southern Saskatchewan in 1982 and subsequently indicated that flixweed, a common cruciferous prairie weed, is the main early season host plant of *N. niger* before this insect moves into developing mustard crops (Burgess pers. observ.). It was also noted that *N. niger* was almost always more abundant in semi-permanent flixweed patches in wasteland, field margins and old farmyards than in flixweed patches in cultivated or cropped fields. Comparison of the annual June abundance of *N. niger* in semi permanent flixweed patches in southern Saskatchewan with the annual

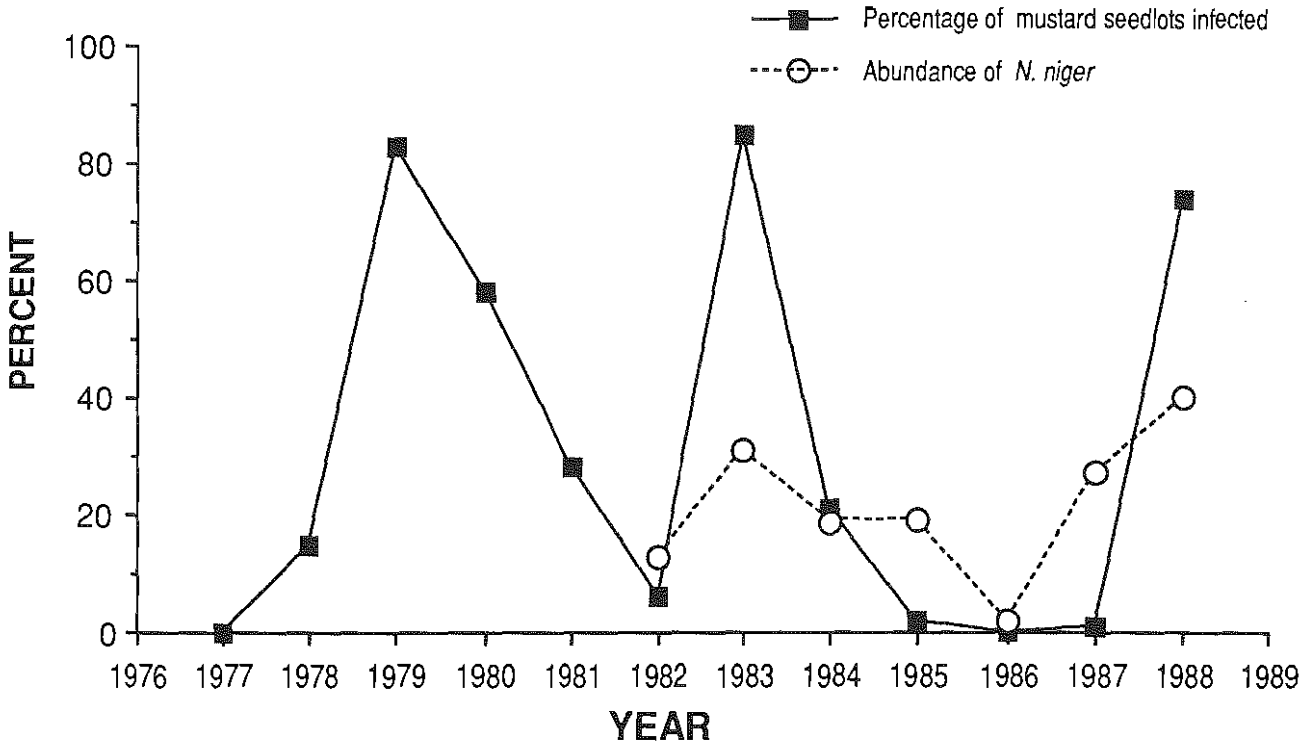

Fig. 1. Percentage of sampled mustard seed lots[1] infected with *Nematospora sinecauda* in the Prairie Provinces – Montana – North Dakota mustard growing area, and June abundance[2] of *N. niger* on flixweed in southern Saskatchewan.
[1] Data include *B. juncea* in all years, and *S. alba* from 1982 onwards, and were obtained by the Mustard Association and Agriculture Canada.
[2] Calculated as the percentage of sites where *N. niger* was abundant in relation to the total number of sites sampled.

percentage of commercial mustard seedlots infected with *Nematospora* in the mustard growing region from 1982 through 1988 (Fig. 1), showed that the two peak years of *Nematospora* infection coincided with the two peak years of *N. niger* abundance. Also, in 1984, when *N. niger* was abundant in flixweed patches in June in southwestern Saskatchewan, but rare in the southeast, the majority of yeast positive mustard seedlots came from southwestern Saskatchewan and southern Alberta. *N. niger* was found in only two of 21 flixweed patches sampled in southeastern Saskatchewan and was abundant in neither, whereas in the southwest it was found in 14 of 18 patches sampled and was abundant in eight. However, the presence of sizable populations of *N. niger* alone did not necessarily produce a *Nematospora* outbreak, as evidenced by the sizable *N. niger* populations recorded in 1985 and 1987 (Fig. 1). The coincidence of the two *Nematospora* outbreaks with peaks of *N. niger* abundance supports the idea that this insect is a major vector of *Nematospora* to mustard crops. Additional evidence of the importance of *N. niger* as a vector was seen in 1988, when yeast positive *N. niger* adults were found on flixweed at four southern Saskatchewan locations in June, whereas no locations with yeast positive *N. niger* had been found in the June survey in the previous two years. These finds were the basis of an accurate alert issued to the mustard industry that an increased incidence of *Nematospora* infected crops could be expected.

While our observations and those reported by Burgess *et al.* (1983) point to the major importance of *N. niger* as a vector of *Nematospora*, some involvement of other insects is not ruled out. *Nematospora* positive western damsel bugs [*Nabis alternatus* Parshley] and *Lygus* bugs were found in southern Saskatchewan in 1981 (Burgess *et al.* 1983) and in 1982, some overwintered *Lygus* adults gave a weakly positive test for yeast [2 colonies/26 insects]. In 1988, four specimens of a stink bug, *Chlorochroa uhleri* (Stal.) that were positive for the yeast were collected from flixweed plants. Stink bugs [Pentatomidae] are well known as vectors of the Nematosporaceae in tropical and subtropical regions (Batra 1973).

Where does *N. sinecauda* inoculum overwinter?

It was first postulated that the yeast might survive the winter in overwintering *N. niger* adults, as it was determined that the yeast remained viable in frozen adults (–10 to –12°C) for more than 12 months, and as *N. niger* has been reported to overwinter as an adult in Alberta (Strickland 1933) and probably also in Saskatchewan (Taylor 1964). Also, Ershad and Barkhordary (1976) found that *N. coryli* overwintered in its insect vectors in Iran. However, our investigations in southern Saskatchewan between 1981 and 1988 yielded no evidence that *N. niger* overwinters here as a nymph or adult. None of 27 samples of leaf litter and turf collected from October to December

1981, near sites where *N. niger* was present earlier in the season, yielded any live nymphs or adults. Each year from 1982 to 1985, a careful early spring examination was made of soil and plant debris at sites where *N. niger* nymphs and adults had been abundant the previous autumn in mustard stubble and beneath unharvested mustard swaths. No evidence of even a single nymph or adult surviving the winter was found, even though densities the previous autumn had sometimes been estimated in thousands per 30 cm square. Placing an emergence cage and additional protective mustard and wheat straw over each of two such aggregations of *N. niger* nymphs and adults in autumn 1984, also produced a negative result. Soil and trash brought into the laboratory from within and around these cages the following spring contained no living adults, and no nymphs until eggs present in these materials hatched a few days later. In annual field observations from 1982 to 1987, including many in flixweed patches, the first *N. niger* nymphs were found between 14 May and 6 June, with the first adults appearing 9-21 days later. Thus it appears that *N. niger* overwinters in the egg stage in southern Saskatchewan, and probably throughout the mustard growing area, as winter conditions in this area tend to be rather uniformly severe. As we could find no published records of yeasts being carried from one generation to the next on or in insect eggs, and Ershad and Barkhordary (1974) found that *N. coryli* was absent from eggs laid by infected adults of the genera *Acrosternum* and *Brachynera*, it seemed unlikely that the yeast overwintered on or in *N. niger* eggs, and other overwintering possibilities for *N. sinecauda* were considered.

The possible reintroduction of the yeast each spring by infected *N. niger* adults migrating into the mustard growing area on winds from warmer, more southerly latitudes was considered. However, the probability that this occurred seemed low. The complete absence of any adult *N. niger* in the study area prior to the appearance of locally developed adults was not suggestive of spring immigration. Also, in the southern states of the U.S.A. that might be expected to be a possible source of yeast positive *N. niger*, if indeed *N. sinecauda* occurs in that area, it has been reported that *N. niger* is replaced by *N. raphanus* Howard (Barber 1947), a species that we did not collect in Saskatchewan at any time during the nine years of the study.

A third possibility was that flixweed, because it is the main early season host of *N. niger*, might serve as an overwintering host for *N. sinecauda* and be the spring source of inoculum for that insect. Evidence that this occurs was obtained when yeast positive *N. niger* nymphs [24 specimens divided into four groups of six; with 4, 56, 60 and 200 yeast colonies per group] were collected on 19 June 1985 from an isolated flixweed patch near Davidson, Saskatchewan. Adults were not yet present at this site, and as nymphs do not fly, it seemed probable that they had acquired the yeast from a source of overwintered inoculum in or near the flixweed patch in which they were found. From late July to early September in the same season, yeast positive adult *N. niger* and additional yeast positive nymphs were found in flixweed patches at various locations, and as well, mature, ripened flixweed seeds collected from two locations proved positive for *Nematospora* [1×10^3 and 2.2×10^4 colonies/g].

With the latter discovery, it was postulated that ripened dormant flixweed seed might be an overwintering vehicle for the yeast. For infected flixweed seed to constitute a spring source of inoculum for *N. niger*, one requirement was that nymphs and adults must feed on the ripened seeds. In July and August 1985, *N. niger* nymphs and adults were observed living on the ground under patches of flixweed in which all plants had senesced and were dry and brittle. This suggested that nymphs and adults, although commonly known as sap suckers in nature, might be feeding on ripened flixweed seeds that had fallen from the pods. This supposition was supported by laboratory feeding tests, in which field collected fourth and fifth instar nymphs and adults fed readily on hard ripened flixweed seeds as well as on ripened canola and domestic mustard seeds. To feed, a nymph or adult grasped a seed between its front tarsi, held it firmly against a paper substrate on the bottom of the test chamber, and drilled deeply into the seed with its mouthparts. As the mouthparts penetrated further into the seed the labial sheath acquired a sharper bend. The time spent feeding on a single seed ranged from about one to more than 30 min. Examination of mustard seeds that were germinated after being fed upon by adult *N. niger* showed that the feeding stylets had penetrated both folded cotyledons, and left areas of brown damaged tissue where no chlorophyll developed.

For overwintered flixweed seeds to be a spring source of *Nematospora* inoculum for *N. niger*, it was necessary also that *N. niger* be able to acquire this inoculum by feeding on *Nematospora* infected seeds. When laboratory reared nymphs and adults were used in inoculum acquisition tests, it was observed that they fed much less readily on ripened flixweed seeds than did their field collected counterparts. In spite of this, two out of five groups of 10 laboratory reared *N. niger* adults became *Nematospora* positive internally [23, 720 colonies/group] after being exposed for 52 h to infected flixweed seed [3.2×10^3 colonies/g]. However, none of 50 fourth and fifth instar nymphs similarly exposed became *Nematospora* positive. Acquisition of *Nematospora* inoculum from infected seed by adults was confirmed in a second test. Five out of 10 groups of five laboratory reared adults of *N. niger* became yeast positive internally [1, 5, 9, 140, 1408 colonies/group] after being exposed to infected seed [7.0×10^7 colonies/g] for 24 hr. Again, none of 24 nymphs similarly exposed became yeast positive. A third experiment, with 50 laboratory reared adults exposed to infected seeds [9.6×10^3 colonies/g] for 48 hours, again confirmed the results of the earlier experiments. Of the 46 adults surviving the experiment, five groups of eight and the remaining group of six had all become yeast positive internally [13, 240, 816, 896, 1696, 2560 colonies/group]. In this experiment adults were permitted to feed also on a greenhouse grown immature *B. juncea* pod for the last 24 hours of the experiment. Plating the seeds of this pod at the end of the experiment yielded 33 colonies of *Nematospora*, while plating of the seeds of two control

B. juncea pods yielded no yeast colonies. Thus it was apparent that *N. niger* adults could acquire *Nematospora* inoculum by feeding on ripened infected flixweed seeds, and transmit it to developing seeds in a healthy mustard pod. The ability of field collected *Nematospora* positive adults to transmit the yeast to the seeds contained in healthy green mustard pods had already been demonstrated by Burgess *et al.* (1983).

A third requirement for overwintered flixweed seeds to be a spring source of *Nematospora* inoculum for *N. niger* was that the yeast must survive the winter in nature in some dormant flixweed seeds. In 1989, it was established that this occurred in a southwestern Saskatchewan flixweed patch whose seed had tested positive the previous September. Ripe dormant seeds, retained over winter within the pods of mature plants in this patch, and collected 19 April, showed a minimum count of 2.1×10^3 colonies of *Nematospora* per gram when plated. Unfortunately, it was not possible to conduct a feeding test with *N. niger* on this seed; however, the level of *Nematospora* infection was of the same order of magnitude as in the seed employed in two of the previous feeding tests when adult *N. niger* acquired *Nematospora* inoculum.

Pod-retained seeds in the spring, in this and other flixweed patches, were most abundant in plants sheltered from the wind by a surrounding dense growth of flixweed or other weeds; the wind had shattered the majority of seeds from isolated plants and those near the patch periphery.

To simulate infected flixweed seeds overwintering on the ground, approximately 4 g of *Nematospora* infected seed [5.7×10^4 colonies/g] was placed on a soft cloth just above the soil surface, as described earlier, in early November 1988. When the seeds were retrieved on 9 May 1989, and subsequently plated, no *Nematospora* was detected. Non survival of the yeast in this seed was perhaps due to the damper conditions existing near the soil surface than in the pods of standing mature plants. Holley and Timbers (1983) have shown that under some conditions, small increases in the moisture content of stored oriental mustard seed reduced *Nematospora* survival.

The foregoing observations indicate that *Nematospora* could overwinter in nature on the western prairies in ripened seeds retained within the pods of mature flixweed plants, and be acquired by *N. niger* adults feeding on these seeds in the following spring. The collection of *Nematospora* positive nymphs on 19 June 1985 suggests that in nature, nymphs also may acquire the inoculum by feeding on overwintered infected flixweed seeds. The failure of nymphs to pick up *Nematospora* in laboratory tests possibly resulted from the low level of seed feeding observed in laboratory reared nymphs.

The possibility of *Nematospora* overwintering in flixweed seedlings was investigated also, since plants arising from seeds germinating in late summer and autumn overwinter in a green vegetative rosette stage. Such plants, collected in the spring from two flixweed patches in 1986 and five patches in 1987, tested negative for *Nematospora*. Both of the 1986 collections came from patches that had infected seed the previous year, and one of the 1987 collections came from a patch that contained infected *N. niger* the previous year. In April 1989, approximately 350 seedings collected from a flixweed patch that had both infected *N. niger* and infected seeds in 1988, and that also yielded infected overwintered seed, likewise proved negative for *Nematospora*. The lack of evidence for overwintering of *Nematospora* in overwintered vegetative flixweed seedlings is not surprising, as the yeasts of the Nematosporaceae are primarily parasites of seeds and fruits (Batra 1973; Preston and Ray 1943; Plurad and Daugherty 1970).

Acknowledgements

We thank the Mustard Association for their strong support of this work, and J.S. Hemingway for information on *Nematospora* infection of mustard crops. H.H. Weegar and J.E. Wiens provided invaluable entomological assistance.

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