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Influence of cyst maturation on apparent population increases of *Heterodera schachtii* on root remnants

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Summary – Three experiments were conducted to study the development of the sugarbeet cyst nematode, *Heterodera schachtii,* on host roots lacking foliage. In a microplot experiment, cabbage-root respiration declined in *H. schachtii*-infected roots that were sequentially sampled after shoot removal. In a greenhouse experiment, the proportion of brown cysts and the number of cysts per g of root increased over time on roots with and without foliage. A third experiment, conducted in growth pouches, revealed that cysts matured and egg number increased on roots in the absence of foliage. Our results reveal that white cysts mature to become brown cysts, egg number per cyst increases, and that juveniles may develop into cysts on roots that are separated from shoots. Thus, sugarbeet cyst nematode numbers may appear to increase in the absence of a host plant, if viable roots are present in the soil. This occurs because cysts and eggs continue to develop, and also because extraction techniques are generally biased toward extracting brown cysts. Consequently, estimates of *H. schachtii* population density may be influenced by time of sampling relative to nematode development on root fragments, and management decisions based on egg-density estimates should account for possible increases in cyst nematode densities occurring on root remnants.

Résumé – *Influence de la maturation des kystes d'*Heterodera schachtii *sur des augmentations apparentes de population dans des racines restées en terre* - Trois expériences ont été réalisées pour étudier le développement des nématodes à kyste de la betterave, *Heterodera schachtii,* sur des plantes hôtes défoliées. Lors d'une expérience en micro-parcelles, la respiration des racines de chou diminue dans les racines infestées par *H. schachtii* prélevées à intervalles réguliers. Lors d'une expérience en serre, la proportion de kystes bruns et le nombre de kystes par gramme de racine s'accroît avec le temps, que les plants soient défoliés ou non. Une troisième expérience, conduite en sacs, a révélé que les kystes mûrissent et que le nombre d'œufs augmente dans les racines de plantes défoliées. Ces résultats démontrent que, sur des racines isolées da la tige, les kystes blancs mûrissent en devenant bruns, le nombre d'œufs par kyste augmente et les juvéniles peuvent éclore à partir des œufs. De ce fait, le nombre de kystes peut sembler s'accroître en l'absence de plante hôte si des racines vivantes demeurent dans le sol. La raison en est que kystes et œufs continuent à se développer et aussi que les techniques d'extraction sont axées sur les kystes bruns. En conséquence, l'estimation de la densité de population peut être influencée par le moment du prélèvement en relation avec le développement du nématode sur les fragments de racines. Les stratégies de lutte fondées sur l'estimation de la densité des œufs devraient donc tenir compte d'une augmentation possible de la densité des kystes due à ceux présents sur les restes de racines.

Key-words : *Beta vulgaris, Brassica oleracea,* cabbage, cyst nematode, cyst maturation, ecology, *Heterodera schachtii,* sampling, sugarbeet, sugarbeet cyst nematode.

Rotation to non-host crops is a successful strategy to reduce densities of *Heterodera schachtii* in infested fields (Steele, 1984). The efficacy of a rotation in reducing nematode densities may be measured as the P_f / P_i ratio, where P_f is the egg density at the end of the rotation and P_i is the egg density at the start of the rotation. The accurate determination of nematode densities is important when selecting, or when assessing the efficacy of, nematode management strategies, particularly in annual crops. If there is an unanticipated increase in actual nematode densities relative to the densities estimated by sampling, the management decision-making process is compromised by inaccurate information.

The population density of *H. schachtii* was observed to increase in weed-free fallow treatments during a microplot experiment conducted in Davis, California, USA (Gardner & Caswell-Chen, 1993). Other researchers have observed similar population increases for *H. glycines* in the field in the absence of a host (T. L. Niblack & R. D. Riggs, pers. comm). Under laboratory conditions, *H. schachtii* has been observed to produce eggs on *Brassica rapa* root explants provided with nutrient solutions (Betka *et al.,* 1991), and *Meloidogyne incognita* females have been observed to produce eggs on cotton and tomato root fragments (Starr, 1993).

An observed increase in egg density in field soils, despite the absence of a host, might arise in two ways : as an actual increase in egg number; or, as an apparent artifact of sampling and extraction methods that underestimate initial egg density relative to the density estimated at a later time. Actual increases in egg density should only occur in the presence of roots that provide adequate resources, such as amino acids (Krauthausen & Wyss, 1982; Betka *et al.,* 1991), for nematode development and egg production. Artifactual increases may occur over time if white cysts that are not recovered

from samples by standard extraction at some time "t" (herein designated P_t) mature to brown cysts that are efficiently recovered from samples taken at some later time, "$t + Dt$" (herein designated P_{t+Dt}). Brown cysts are efficiently recovered by our standard method of processing cysts to release eggs (Caswell *et al.*, 1985). This method relies on drying the sample to extract the cysts; however, during drying white cysts containing eggs desiccate and collapse and are not subsequently recovered. If P_t samples are taken before, or simultaneously with, the removal of host foliage, and white cysts on roots continue development into brown cysts during the interval D_t, then later sampling and successful extraction of the brown cysts to establish P_{t+Dt} would indicate increased egg numbers and the ratio of egg numbers of later (P_{t+Dt}) to earlier (P_t) samples would reveal a population increase $([P_{t+Dt}/P_t] > 1)$.

We considered three nested hypotheses that possibly explain a population increase despite the absence of a complete host plant, occurring over a time period (D_t) defined by an initial population estimate (P_t) and a final population estimate (P_{t+Dt}):

Hypothesis 1: The number of brown cysts increases on root fragments during D_t. When the P_t soil sample is taken, brown cysts and white cysts containing eggs are present. In the soil, during the interval D_t, white cysts containing eggs mature to brown cysts, but additional eggs are not produced. The number of brown cysts recovered from a later sample, the P_{t+Dt} soil sample, is greater than that recovered from the P_t sample. Thus, P_{t+Dt}/P_t is greater than one, and the observed increase in egg numbers results from the increased number of brown cysts in the P_{t+Dt} sample and not from an increase in total cyst or egg numbers.

Hypothesis 2: The number of brown cysts and the number of eggs per cyst increase on root fragments during D_t. The P_t sample is the same as suggested in hypothesis 1. During the interval white cysts on roots produce more eggs and eventually mature into brown cysts. The observed increase in egg number $([P_{t+Dt}/P_t] > 1)$ results from the increased number of brown cysts that were extracted at P_{t+Dt}, and the increased number of eggs per cyst.

Hypothesis 3: Juvenile stages mature into white cysts and produce eggs, and the number of brown cysts and the number of eggs per cyst increase on root fragments during D_t (same as hypothesis 2). The P_t is as in hypotheses 1 and 2. After sampling, some juveniles, early adults, and white cysts continue their development, produce more eggs, and eventually mature into brown cysts. The $(P_{t+Dt}/P_t) > 1$ because brown cysts, total cysts, and egg numbers have all increased during D_t.

Although these three hypotheses are not mutually exclusive, the three experiments presented here were conducted to test the elements of these hypotheses. The experiments were designed to evaluate root respiration (as an estimator of root viability), maturation of white cysts to brown, egg production, and recruitment of juveniles into adult stages.

Materials and methods

Experiment 1

This experiment was conducted to observe root fragment health, as measured by root respiration, and cyst maturation on decapitated cabbage roots. The experiment was conducted in established microplots consisting of five 208-liter plastic drums with open tops and perforated bottoms, buried to ground level. The bottoms were filled with an 8-cm layer of gravel, and the remainder filled with river sand (94 % sand, 3 % silt, 3 % clay). The microplots had been infested with *H. schachtii* (from greenhouse culture on beet, originally obtained from Half Moon Bay, CA) 2 years previously, and had had sugarbeets and cover crops in them since that time (Gardner & Caswell-Chen, 1993). Three cabbage plants (*Brassica oleracea* L. var. *capitata* cv. Copenhagen Market) were planted into each microplot. After 5 months, the foliage was removed and the microplots were sampled weekly by taking three 2.5 cm diam. × 30 cm cores. Each core was removed from a previously unsampled area. All roots and attached cysts recovered from the samples were rinsed free of sand on a 2 mm-pore sieve, gently blotted dry, weighed, and placed in a Gilson differential respirometer (Gilson Medical Electronics Inc., Middleton, WI, USA). Roots were subject to visual inspection, and white roots were considered healthy and brown roots were considered unhealthy. A 0.1 % aqueous solution of 20-20-20 fertilizer (Gro-More Inc., Gardena, CA, USA) was used to moisten the roots in the reaction vessels. Reaction vessles were continually agitated in a water bath at 21 °C, and total oxygen uptake in the vessel was measured for 60 min. Root respiration was used as a measure of the overall activity of the entire sampled root mass (weight range 0.03 to 0.42 g). Roots were then removed from the reaction vessel and sandwiched between glass plates and the numbers of brown, slightly brown, and white cysts were counted at 40 × magnification. White and slightly brown cysts were considered immature and data were pooled as white cysts. The change in brown cysts per g of root and of brown cysts as a proportion of total cysts was analyzed by least squares regression. There were no untopped controls for this experiment. Weekly respiration and cyst data were compared with respiration and cyst data taken at the time of foliage removal.

Experiment 2

This experiment was conducted to assess cyst development on roots after removal of foliage. Cabbage (cv. Copenhagen Market) seeds were sown in ten 58-liter pots (41-cm diam.) filled with washed mortar sand and maintained in a greenhouse. After 14 days, plants were

thinned to three plants per pot. On days 18, 43, and 57, all pots were infested with *H. schachtii* J2 by pipetting a suspension onto the sand at the base of each plant. Plants were grown for 136 days before foliage was removed from the cabbage in five of the ten pots. At that time and for 5 weeks following the decapitation, pots were sampled six times at approximately 3-day intervals by taking three cores (2.5 cm-diam. × 20 cm) per pot, for a total of eighteen cores. Cores were removed from areas undisturbed by previous sampling. Roots were rinsed free of sand on a 2.0 mm-pore sieve and any escaping small roots and cysts were caught on a 0.246 mm-pore sieve. Roots and cysts from both sieves were combined and weighed. Root respiration and enumeration of brown, slightly brown, and white cysts was performed as described for Experiment 1. The experiment was conducted twice (referred to as trials 1 and 2).

Cyst data from the greenhouse trials were analyzed by least squares regression of cysts per g of root and brown cysts (as a proportion of total cysts), on time after topping. Proportion data showed homogeneity of regression for like treatments between trials, so data were pooled. Tests of significance for regression and correlation coefficients from pooled proportion data were performed using t-tests (Edwards, 1984). Homogeneity of regressions for topped and untopped treatments using pooled proportion data was tested using F-tests. The respiration values and trends were also similar for both trials, so those data were pooled. Respiration data were included for untopped controls as a basis for comparing topped treatments. The data for cysts per g of root were not combined.

Experiment 3

This experiment was conducted to quantify cyst color change and egg production on host roots lacking foliage. Twenty five seeds of sugarbeet *(Beta vulgaris)* cv. SS 334 (Spreckels Sugar Co.) were sown in growth pouches (CYG Growth Pouch, Mega International, Minneapolis, MN, USA) and allowed to grow until roots approached the bottom of each pouch. All pouches were then inoculated with approximately 900 J2 of *H. schachtii* recovered from cysts placed on Baermann funnels. The J2 were obtained from greenhouse cultures maintained on sugarbeet. Pouches were maintained on the laboratory bench at approximately 25 °C, under four fluorescent white lights (F40CW) at a distance of 50 cm, irrigated daily with distilled water and weekly with a complete nutrient solution (Lambert *et al.,* 1992). Excess water was drained from the pouches after each irrigation.

The inoculated seedlings were grown until white cysts and a few slightly brown cysts were visible on the roots (38 days in trial 1, 40 days in trial 2). At that time, the pouches were divided into four groups. One group was used to determine mean number of eggs per cyst before treatment. Fifteen white cysts per pouch were removed and ground in a tissue homogenizer (25-100 Lambda Econo-Grind Homogenizer, Radnoti Glass Technology Inc., Monrovia, CA, USA) to release eggs. The mean number of eggs per cyst before treatment served as a baseline for subsequent comparison of the three remaining treatments, *i*) topped (foliage removed), *ii*) topped + glyphosate (1 Roundup™ [41 % a.i.] : 9 distilled water applied to the remaining hypocotyl with a cotton swab), and *iii*) untopped. The topped + glyphosate treatment was included to produce an environment that would deprive the nematodes of a food source more rapidly than would topping alone.

Concurrent with determining baseline eggs/cyst, the locations of fifteen individual white cysts were marked on the outside of each pouch of the remaining three treatments. In this manner, individual cysts were monitored and their color change recorded during the experiment. When the rate of change from white to brown cysts had slowed and stabilized, the experiment was terminated and the mean number of eggs per marked cyst was determined using the method described previously. The experiment was conducted twice. Differences in mean eggs/cyst between treatments and control were determined using Dunnett's test, and differences among treatments were determined using Duncan's means separation (Steele & Torrie, 1980).

Results

The three experiments addressed several aspects of cyst nematode development on root fragments, accordingly, the results are presented not by experiment, but rather as they regard : root fragment respiration; maturation of white cysts; continued egg production on root fragments; and, the recruitment of new cysts from J2, J3, or J4 stages.

Root respiration

In Experiment 1, roots that appeared healthy were present throughout the duration of the experiment. Root respiration declined rapidly but stabilized at approximately 20-25 % of initial values after 13 days (Fig. 1).

In Experiment 2, healthy roots were present for the duration of the experiment. Root respiration by topped plants declined rapidly over 5 days but declined more slowly after that time. Respiration by untopped controls increased relative to initial values, but had declined below initial values by 25 days after topping (Fig. 1).

Maturation of white cysts

In Experiment 1, total cysts per g of root declined initially but stabilized after approximately 15 days (Fig. 2A). The number of brown cysts/g of root was variable over time and non-linear. The proportion of brown cysts relative to total cysts increased as a natural growth function ($P \leq 0.01$, $r^2 = 0.99$) so that essentially all cysts were brown by approximately 25 days (Fig. 2B).

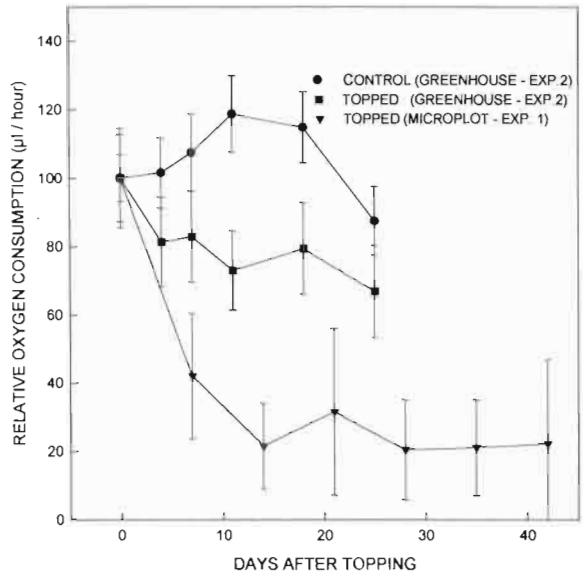

Fig. 1. *Oxygen consumption as a percentage of initial values for greenhouse and microplot experiments. Error bars represent relative uncertainty determined by Kline-McClintock Theorem (Moffat, 1985).*

In Experiment 2, the number of brown cysts per g of root increased ($P = 0.038$ and 0.002 for trials 1 and 2, respectively) on topped treatments. White cysts/g root declined ($P = 0.0062$ and 0.017 for trials 1 and 2, respectively) and regression slopes for intermediate colored cysts were not different from zero (Fig. 3). Data are not presented for untopped controls. Using pooled data, the proportion of brown cysts from topped treatments increased ($P = 0.006$) over time (Fig. 4). Regression slopes were different ($P \leq 0.05$) for topped and untopped treatments; the slope for the topped treatment was greater than for the untopped treatment.

In Experiment 3, cyst color changed from white to brown in all treatments. The percentage of brown cysts from fifteen marked cysts increased with time while the number of white cysts declined. Cysts of intermediate color increased but leveled off (Fig. 5). The change from white to brown was most rapid for the topped + glyphosate treatment and slowest for the untopped treatment.

Egg production

In Experiment 3, egg production by white cysts continued on beet roots after topping. In the first trial, egg production was greatest on untopped beets, but both topped and topped + glyphosate treatments had greater egg numbers than did the pre-topping controls (Fig. 6). In the second trial, there were no significant differences between pre-topping controls and other treatments (Fig. 6).

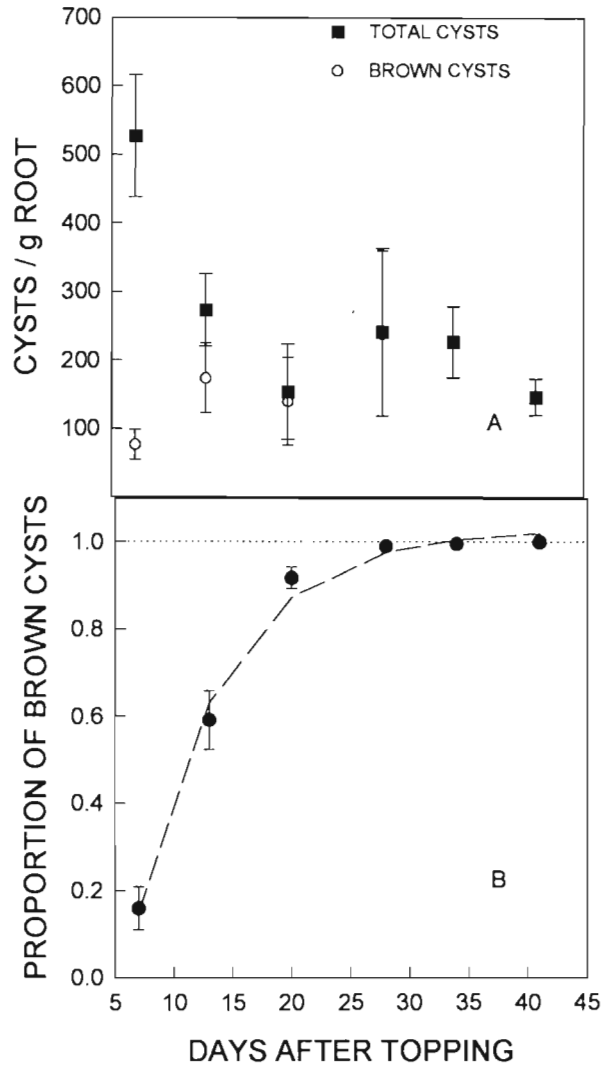

Fig. 2. *A : Brown and total cysts/g of cabbage root; B : Brown cysts as a proportion of total cysts. Data are from Exp. 1 conducted on decapitated cabbage roots in microplots. Error bars are one standard error. Significant regression ($P < 0.001$) shown.* $Y = (2.22\text{-}2.22e^{(-0.13X)})\text{-}1.2$ ($r^2 = 0.99$).

Cyst recruitment

In Experiment 1, there was a decline in total cysts per g of root (slope = – 8.18 cysts/g root/day, $P = 0.007$) during the experiment. (Fig. 2A).

In Experiment 2, total cysts per g of root increased (slope = + 1.04 cysts/g root/day, $P = 0.038$) for topped treatments in trial 1. For trial 2, total cysts/g root increased ($P = 0.044$) for 18 days, but declined after that time (Fig. 3). Because the total number of cysts (white + intermediate + brown) increased, the recruitment of juvenile stages into the cyst pool was inferred.

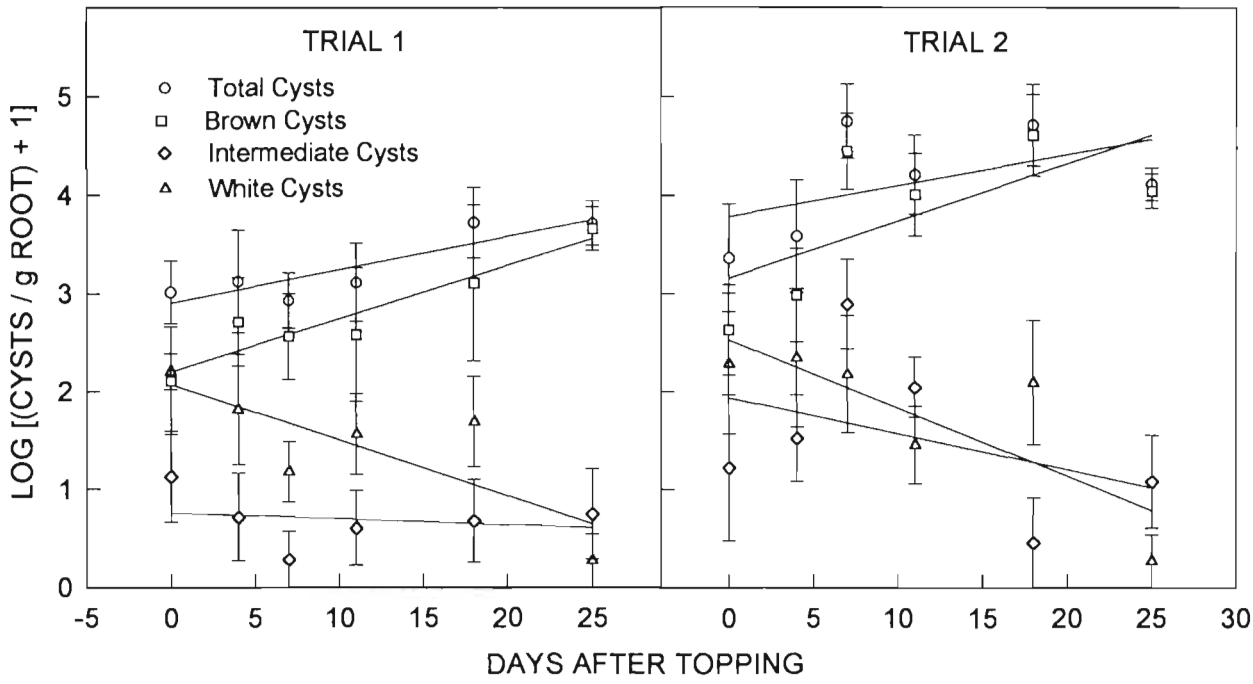

Fig. 3. *Total cysts/g of root, and brown, white, and intermediate colored cysts/g of root from topped cabbage in Exp. 2 conducted in 58-liter pots in greenhouses. Error bars are standard errors. Regression slopes were significant and positive for total and brown cysts, significant and negative for white cysts, and no different than zero for intermediate colored cysts. The experiment was conducted twice (Trials 1 and 2).*

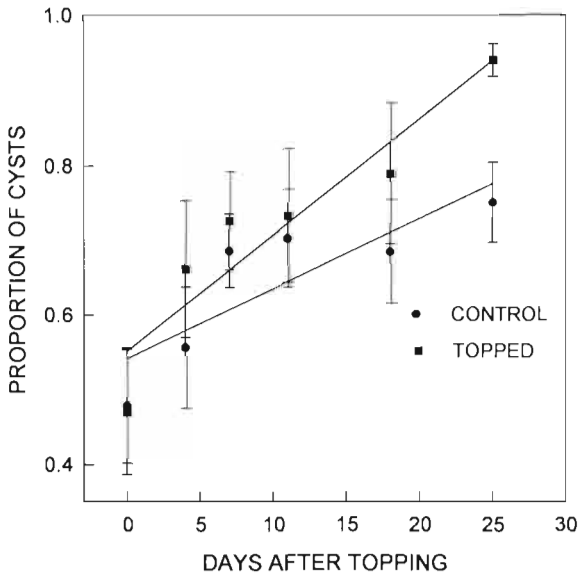

Fig. 4. *Brown cysts as a proportion of total cysts on cabbage roots from Exp. 2 conducted in 58-liter pots in greenhouses. The experiment was conducted twice, and the data were pooled. Regression slopes significant and different ($P \leq 0.05$).*

Discussion

Our research shows that juvenile sugarbeet cyst nematodes on decapitated host roots can produce eggs and eventually become mature cysts. This phenomenon, combined with our standard sampling methods, may result in misleading estimates of cyst nematode egg densities. The data reveal that the several phenomena operating within our third hypothesis are possible, and thus several simultaneous factors result in an apparent increase in *H. schachtii* egg numbers on root fragments.

This is in general agreement with previous research showing that young seedling roots, decapitated by removing root tips and shoots, supported *H. schachtii* development from the J2 to the female stage provided they were given a supplemental nutrient solution that contained minerals and sucrose (Betka *et al.,* 1991; Grundler *et al.,* 1991). It is possible that the older roots examined in our experiments had sufficient nutrient reserves that they were capable of supporting continued female development without supplemental minerals or sucrose.

Roots from the microplot and greenhouse studies continued to respire and some of the roots appeared healthy long after topping. Our observation of cyst maturation and continued egg production on these decapi-

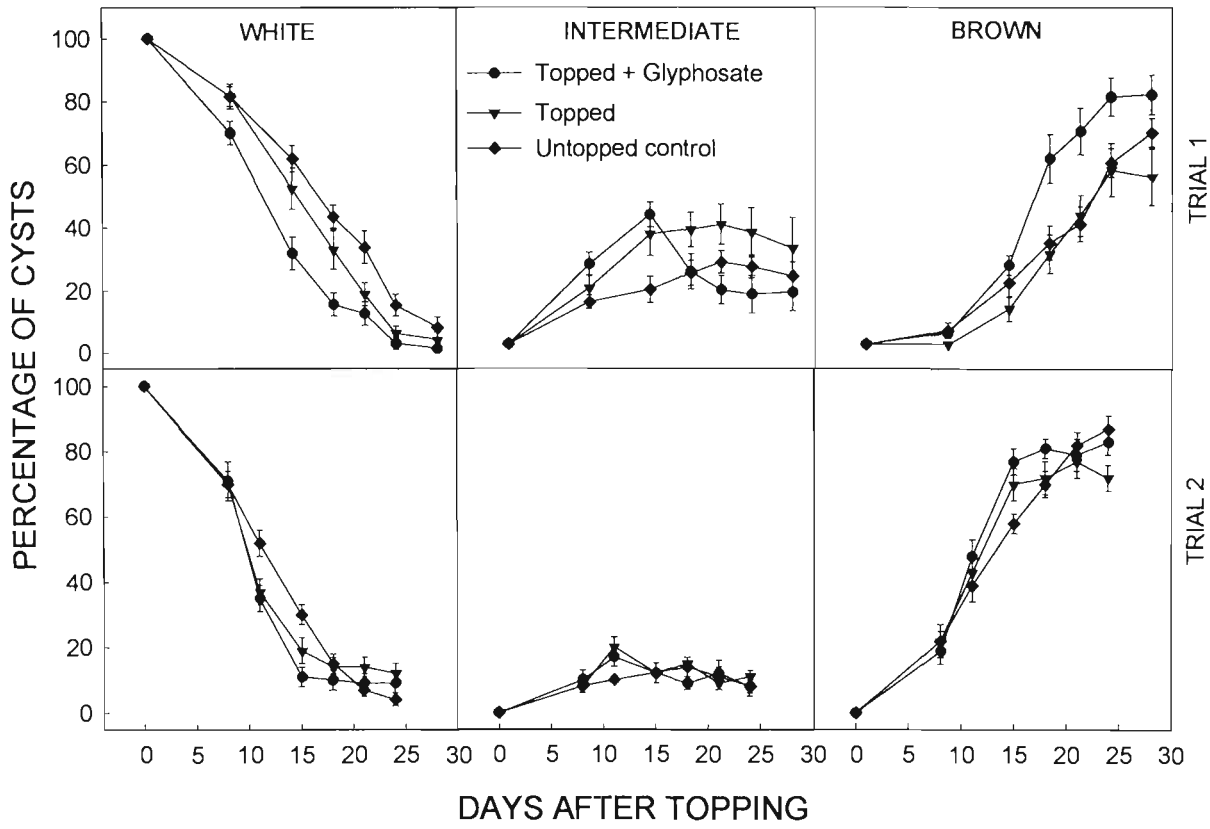

Fig. 5. *White, intermediate, and brown colored cysts as a percentage of fifteen marked cysts (Exp. 3) conducted in growth pouches. The experiment was repeated twice (Trials 1 and 2). Error bars are standard errors.*

tated but actively respiring roots reveals that they were capable of supporting nematode development. We recognize that some of the O_2 consumption observed in our experiments may have been microbial, but visual inspection suggested that healthy roots were still present 25 days and 5 weeks after foliage removal in the greenhouse and microplot experiments respectively. In Experiment 2, root respiration for the topped treatment declined rapidly compared to untopped controls (Fig. 1) indicating that root health declined because of foliage removal. The drop in root respiration for untopped controls after 18 days may be due, in part, to cumulative root damage caused by our sequential, destructive sampling procedure. Further research is necessary to determine if cyst maturation rate is affected by root disturbance while foliage is intact.

An increased number of brown cysts per g of root and an increase in the proportion of brown cysts indicates that cysts can mature on roots without foliage. In Experiment 1, brown cysts as a proportion of total cysts increased while total cysts per g of root declined (Fig. 2A, B). In this situation, the increasing proportion of brown cysts could result from a loss of white cysts during sample processing. Thus, the use of brown cysts per g of root may, in general, be a more appropriate measure of cyst maturation since a loss of white cysts will cause a proportional increase in brown cysts. In Experiment 2, total cysts per g increased (Fig. 3), so in this case, proportions can be used to measure cyst maturation (Fig. 4). In Experiment 2, regression slopes were positive and different ($P \leq 0.05$), indicating that the proportion of mature cysts increased over time. Interestingly, the rate of change of cyst color was accelerated by foliage removal (Figs 4, 5). In Experiment 2, cyst maturation was more rapid in topped treatments than in controls (Fig. 4). The trend was corroborated in the pouch experiment where cysts from topped and topped + glyphosate treatments showed a more rapid transition from white to brown than did cysts in the untopped treatments (Fig. 5). The cause of accelerated maturation is unknown; perhaps healthy root remnants are capable of supplying sufficient nutrition, but topping decreases the nutrients such as amino acids and sugars necessary for continued female development (Betka *et al.*, 1991). In the growth pouch experiment, where the number of counted cysts was constant, the proportion of brown

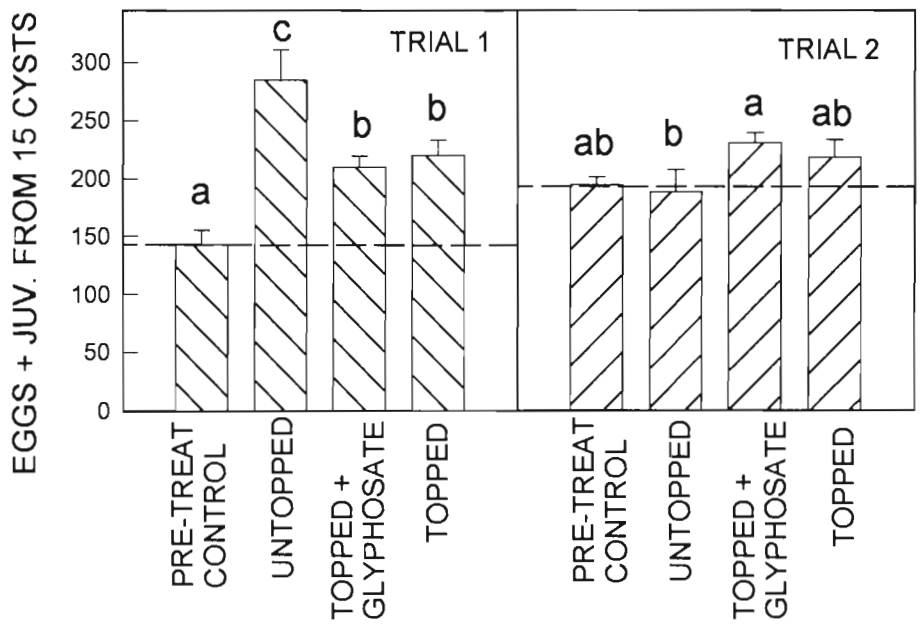

Fig. 6. *Eggs and juveniles from fifteen cysts on sugarbeets grown in pouches for approximately 25 days after foliage was removed from the plants. The experiment was repeated twice (Trials 1 and 2). Error bars are standard errors. Unlike letters within trials are different (P ≤ 0.05) according to Duncan's means separation.*

cysts increased over time while the proportion of white cysts declined. For cysts of intermediate color, the proportion of cysts increased and then stabilized, presumably because the rate of change from white to intermediate cysts was approximately equal to the rate of change from intermediate to brown cysts (Fig. 5). The observed change from white to brown cysts corroborates the results from the greenhouse experiments (Fig. 3) and lends further support to the hypothesis that white cysts can mature into brown cysts in the absence of host foliage.

The data from growth pouch experiments were somewhat variable, but do lend support to hypothesis 2, indicating that it is possible that egg numbers can increase on host roots detached from host foliage. In one trial we did observe a significant increase ($P \leq 0.05$) in egg numbers for all treatments, relative to pre-treatment controls (Fig. 6). In the second trial of the experiment, the lack of significant differences from pre-treatment controls for the other treatments might be explained by egg hatch within the pouches. In the untopped treatment, if there was substantial egg hatch before the counts were conducted, the mean eggs per cyst could be lower than anticipated. Regardless, our results from the first replication of the experiment clearly indicate that continued egg production is possible.

Given sufficient time and nutrition, cyst recruitment from juvenile stages can occur on host roots devoid of foliage. Although we could not show recruitment in Experiments 1 and 3, it is fairly conclusive for Experiment 2, because the total number of cysts increased and the only way that could have occurred was through recruitment of juveniles into the white cyst stage. The data do support the third hypothesis. In Experiment 1, there was a decline in total cysts per g of root, indicating a net loss of cysts. Since Experiment 1 was conducted outdoors in exposed soils, cyst losses due to predation and parasitism were possible. In Experiment 3, the root mass of topped treatments was low and probably insufficient for many juveniles to mature. However, in Experiment 2, total cysts per g of root increased, indicating that juveniles had matured and were visible as adults on the roots of topped treatments. The results suggest that the level of cyst recruitment from juvenile stages will depend on the dynamics of the host-parasite interaction, and probably on the nutrient status of the root fragments (Betka *et al.,* 1991; Grundler *et al.,* 1991).

To conclude, cysts can mature, their numbers increase, and egg production can continue on respiring excised roots. The ramifications are manifold. Sampling time may influence cyst-nematode egg counts if sampling and extraction methods are used that recover only mature, brown cysts. Therefore, in standard sampling and extraction for nematode cysts at the end of a growing season, a sufficient period of time should elapse after crop removal to allow maturation of white cysts to the brown stage. If the sampling and extraction procedure is conducted while viable root fragments remain in the soil, egg counts may be artificially low relative to what would be observed if samples were collected later. If techniques

are used that recover white cysts, then it will be necessary to determine if egg number is affected by cyst development and maturation subsequent to the initial sampling. The phenomena described in this paper is important in research to quantify damage thresholds, and where great accuracy is necessary, *viz.* when sampling for infestation levels at or near the economic damage threshold. From a practical standpoint, if nematodes continue to develop or increase on detached roots, rotation crops intended to manage nematodes may need to be removed earlier than expected to prevent unanticipated reproduction. In California, crop rotations of long duration have sometimes been insufficient to control sugarbeet cyst nematode, and nematode reproduction on root fragments may be involved. If weed hosts are permitted to establish large root systems, cultivation may not eliminate nematode reproduction. Weed management should be implemented prior to weed establishment.

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