Hatching and Development of the Cereal Cyst Nematode, *Heterodera avenae*

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Authorization to Submit Thesis

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Abstract

The cereal cyst nematode, *Heterodera avenae* Woll is an important nematode on wheat in Idaho and causes significant losses in production in infested fields. Hatching behavior is a key factor in the infection process of *H. avenae* and is primarily driven by temperature. Cold soil temperature is required to break dormancy and induce hatching. Hatching of *H. avenae* second stage juveniles (J2) was induced by extended periods of low soil temperatures. Both dormant eggs and encysted eggs were induced to hatch after exposure to 4℃. Hatched (J2) and developed white females from dormant eggs increased at 4℃ with increasing storing periods from two to eight weeks. No hatching of J2 and development of white females occurred when dormant encysted eggs were stored at 20℃ for any storing periods. The recorded hatching percentages of J2 of *H. avenae* were low and did not exceed 11%. The developed white females were found on the wheat roots 9 weeks after emergence of seedlings. Inducing hatching of dormant eggs and encysted eggs of *H. avenae* by low temperature exposure provides an alternative source of inoculum to naturally infested soil.

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Dedication

I dedicated this thesis to the memories of my beloved father and young brother, praying that Allah Almighty keeps them in peaceful glory of the garden of Paradis.

I also dedicated it to my wife, Hanan, to whom I am gratefully indebted for her support in the hardest time of her life.

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Chapter 1: *Heterodera avenae*: Literature review and research objectives

Distribution of *Heterodera avenae*

The cereal cyst nematode, *Heterodera avenae* Woll. (1924) is an important plant parasitic nematode that causes economic yield reduction on wheat worldwide. *H. avenae* has been reported on wheat from diverse climatic regions across the world including Australia (Brown, 1984), West Asia (Sikora, 1988; Al-Yahya et al., 1998), India (Khan et al.,1990; Singh et al., 2009), Europe (Rivoal and Cook, 1993), Saudi Arabia (Youssif, 1987), Egypt (Ibrahim et al., 1986; Ibrahim and Handoo, 2007), and in North Africa including Libya, Tunis, and Morocco (Sikora, 1988). In 1974, *H. avenae* was first detected in Oregon in the United States (Jensen et al., 1975). In Idaho, *H. avenae* was first reported on barley in 1985 (Hafez and Golden, 1985). Figure (1.5).

Wheat production in Idaho

According to a 2020 press release by National Agricultural Statistics Services, Idaho produced 1.8 million tons of winter wheat and 1.2 million tons of spring wheat (National Agricultural Statistics Services, 2020). In eastern Idaho where heavy infestations of *H. avenae* are common, wheat is often produced as a 2-year monoculture of winter wheat (10 months) and fallow (14months), or as a 3-year rotation of winter wheat, spring wheat or barley, and either a pulse crop or fallow conditions (Smiley et al., 2011).

Economic Impact of *Heterodera avenae*

Despite only having one generation per growing season, *H. avenae* can cause a considerable yield reduction in wheat. Yield losses reached 90% in severely infested fields in

Australia and some other temperate regions (Rivoal and Cook, 1993; Riley and McKay 2009). Yield losses due to *H. avenae* on wheat are reported to be 40-92% in Saudi Arabia (Ibrahim et al., 1999), 10% in China (Peng et al., 2009), and 40-50% in Morocco (Rammah, 1994). In eastern Idaho where *H. avenae* is spread in the counties of Jefferson, Madison, and Fermont, the yield loss was more than 70% due to the damage caused by *H. avenae* (Hafez and Golden, 1985). In eastern Oregon, a 50% reduction in winter wheat yields was documented in heavily infested fields (Smiley et al., 1994). Wheat growers had to undergo extreme crop rotation over 6 to7 growing seasons with non- hosts to reduce the population density of *H. avenae* to below the damage threshold of 5 eggs/g soil in eastern Oregon (R. Smiley, personal communication). Figure (1.1) is an aerial view of a wheat field infested with *H. avenae* in Victoria, Australia.

Figure 1.1. Aerial view of a wheat field infested by the cereal cyst nematode, *Heterodera avenae*. Light-colored areas are regions of poor growth due to heavy infestation on wheat (Mactode Publications, Mactode Publications, Bugwood.org. Picture number 5441817).

The annual reduction in wheat yield in Idaho, Oregon, and Washington due to *H. avenae* was estimated to be about 21000 tones valued at US$3.4 million in early 2000's (Smiley and Nicole, 2009; Smiley et al., 2005).

Biology and life cycle of *Heterodera avenae*

Nematodes are microscopic, unsegmented worms. Plant-parasitic nematodes have a unique structure due to the presence of a stylet, which is used for the penetration of plant roots. They migrate inter or intra cellular inside the plants. The cereal cyst nematode, *H. avenae* has only one generation per growing season and a range of 200 to 500 eggs are retained within the female's body (about 700µm in length) up to 6 years (Baldwin and Mundo-Ocampo, 1991). The eggs pass through embryogenesis and the final stage of embryogenesis produces a first-stage juvenile (J1). The J1 is retained in the egg and molts into the second-stage juveniles (J2) also within the egg (Smiley et al., 2017). At the end of growing season or under unfavorable conditions when white female dies, the body wall hardens to form a brown cyst. Freshly hatched J2 release and penetrate epidermal cells behind the root cap of host plant, move intracellularly to the growth zone, and reach the differentiating vascular cylinder where they select a competent cell to initiate and induce a feeding site (syncytium). Syncytium development is induced when pharyngeal gland secretions are injected through the stylet (Smiley and Yan, 2010). The syncytium provides a food resource for the developing nematode. Second stage juveniles molt to the third-stage juveniles (J3) which is sedentary and molt to the fourth stage which is differentiated to females or males. Different stages of the life cycle of *H. avenae* are shown in figure 1.2.

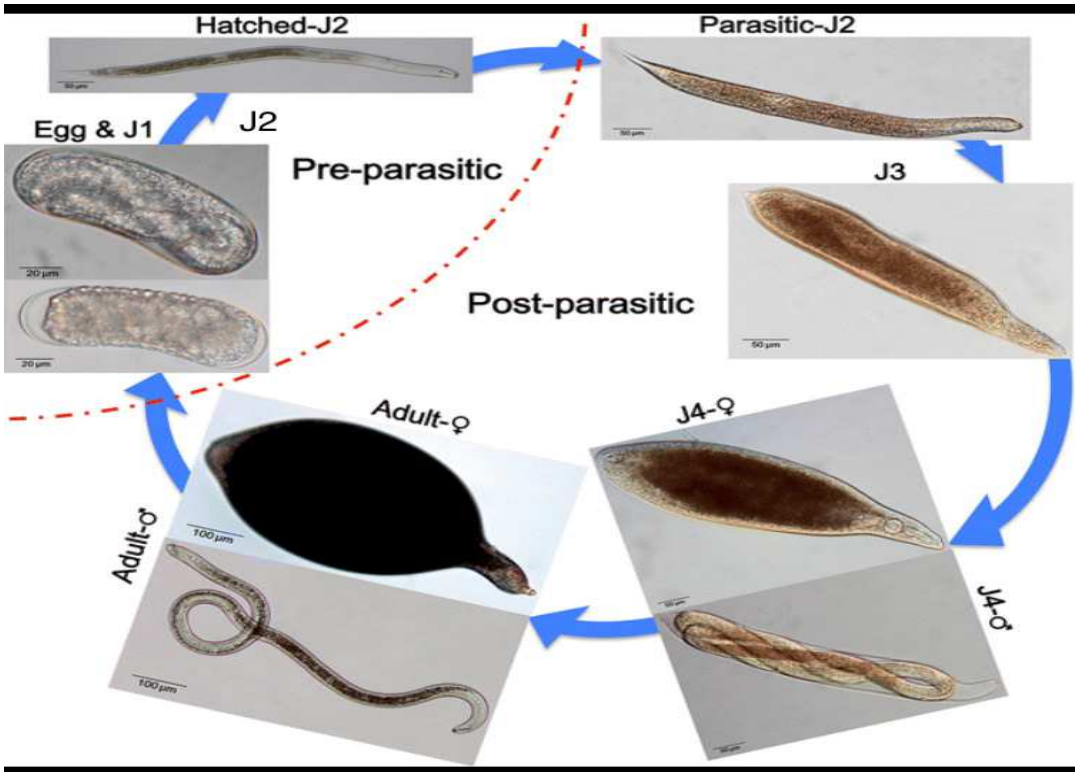


Figure 1.2. Development stages of *Heterodera avenae*. (Yang et al., 2013).

Symptoms and damage threshold of *Heterodera avenae*

Heavy infestation of *H. avenae* results in galls on wheat root (Figure 1.3) usually with some white females attached to these galls (Rivoal and Cook, 1993). Plants with heavily damaged roots may be severely stunted and may mature early (Smiley and Yan, 2010). Symptoms become more pronounced when the infected plants are also exposed to a stressor, such as inadequate nutrition, shallow soil, or shortage of available water (Figure 1.4).

H. avenae disturbs several physiological aspects of infected wheat plants, such as photosynthesis, mineral uptake, transpiration, temperature of the plant canopy by exhibiting temporary wilting, and water content of leaves and roots (Al-Yahya et al., 1998). The J2 and white females of *H. avenae* secrete different enzymes and proteins into host cells for compatible and successful parasitism. These secretions include cell wall degrading enzymes

and effectors which induce the formation of syncytium, as well as suppressors of plant defense system (Ali et al., 2017). The cyst-forming nematodes secrete an annexin like gene and expansins that mimics plant annexin function during parasitic interaction (Patel et al., 2010; Sampedro and Cosgrove, 2005). The annexins are involved in a variety of defense mechanism in plants (Patel et al., 2010). The expansins control the cell enlargement and loosening of plant cell walls that occur as part of the syncytium development (Sampedro and Cosgrove, 2005). Annexins and expansins are the most recent discovered effectors in *H. avenae* and silencing these genes in wheat reduced the infectivity of *H. aveane* (Chen et al., 2015; Liu et al., 2016). The damage threshold of *H. avenae* varies from region to region depending on factors such as soil type, rainfall, cereal crop, or cultivar, nematode pathotype and soil nutrients (Rivoal and Cook, 1993; Simon, 1980; Williams and Beane, 1982). In Saudi Arabia, the damage threshold was seven juveniles per gram of soil on the susceptible cultivar Yecora Rojo (Al-Hazmi et al., 1999). In India, this number was six eggs per gram of soil (Swarup and Sosa-Moss, 1990). In the Pacific Northwest (PNW), the damage threshold of *H. avenae* exceeds five nematodes per gram soil (Smiley and Yan, 2010).

Figure 1.3. *Heterodera avenae* produce knotting on wheat root (Vanstone, 2008)

Figure 1.4. *Heterodera avenae* causes patches of yellowed and stunt plants. Note the likeness of symptoms to poor nutrition or water stress (Vantson, 2008).

Identification of *Heterodera avenae*

The cereal cyst nematode complex consists of six species that are parasitic to cereal crops (*H. australis, H. avenae, H. filipjevi, H. hordecalis, H. latipons, and H. sturhani*) (Smiley et al., 2017). In the PNW both *H. avenae* and *H. filipjevi* are found in small grain fields, but *H. avenae* is the most widespread species (Figure 1.5). Pure populations of either *H. avenae* or *filipjevi* have been found in infested fields in the state of Washington and Idaho (Smiley, 2016). Fortunately, no pathotypes of *H. avenae* have been recorded in the PNW (Smiley et al., 2011). The pathotypes of *H. avenae* are distinguished by testing unknown populations to 'The International Test Assortment for Defining Cereal Cyst Nematode Pathotypes,' suggested by Andersen and Andersen (1982) and updated by Rivoal and Cook (1993) which determines three primary virulence populations of *H. avenae* based on host resistance reaction to a matrix of barley, oat, and wheat cultivars.

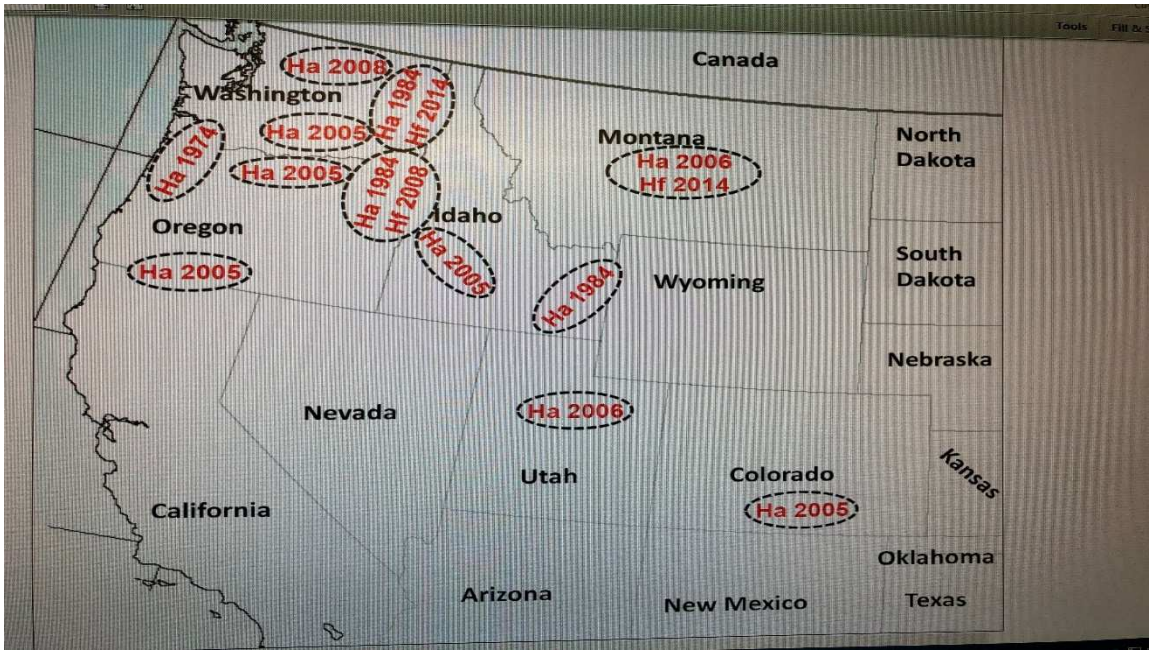


Figure 1.5. Year in which *Heterodera avenae* (Ha) and *Heterodera filipjevi* (Hf) were first reported in regions of the Western United States (Smiley, 2016).

Features such as vulval underbridge, fenestra, semifenestrae, bullae, vulval slit, stylet, hyaline tail tip, and lateral field are well described (Baldwin and Mundo-Ocampo, 1991; and Subbotin et al., 2003). Identification of J2 (Figure 1.6) usually requires a combination of the following morphological characteristics: body length, stylet length, tail length, length of hyaline part of the tail, stylet knob shape, and shape of tail terminus (Sturhan, 1982; Subbotin et al., 2003).

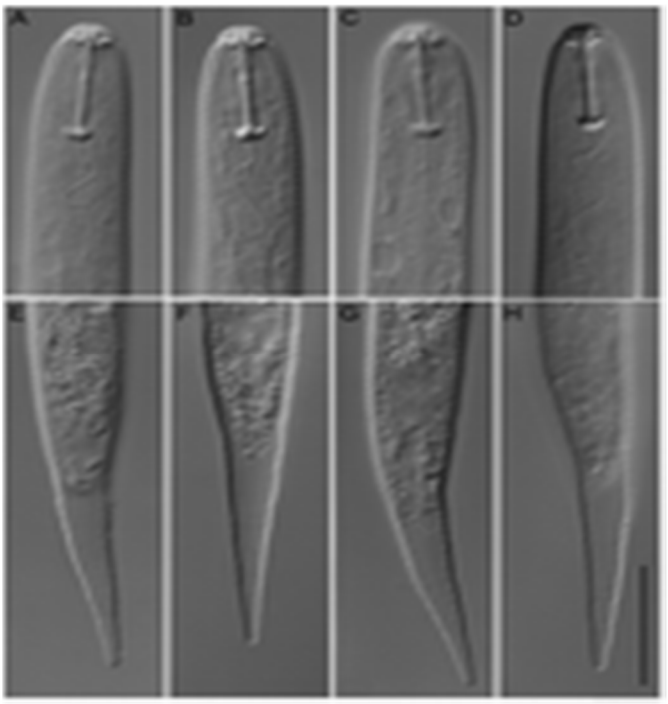


Figure1.6. Anterior and posterior ends of the second-stage juveniles of species belonging to *avenae* group. A, E, *H. avenae*; B, F, *H. australis*; C, G, *H. filipjevi*: D, H, *H. sturhani*. Scale bar = 30 µm (after Subbotin et al., 2003).

Morphological identification of cysts of the different species of the cereal cyst nematode *Heterodera* is based on shape, color, cyst wall pattern, fenestration, vulval slit length. The most important characteristic is the presence or absence of bullae and underbridge of the posterior cone (Mulvey, 1972; Handoo, 2002). Circumfenestrate, bifenestrate and ambifenestrate are the most common types of fenestration (Figure 1.7). The vuval underbrdige is absent in *H. avenae* and present in *H. filipjevi* (Smiley, 2016).

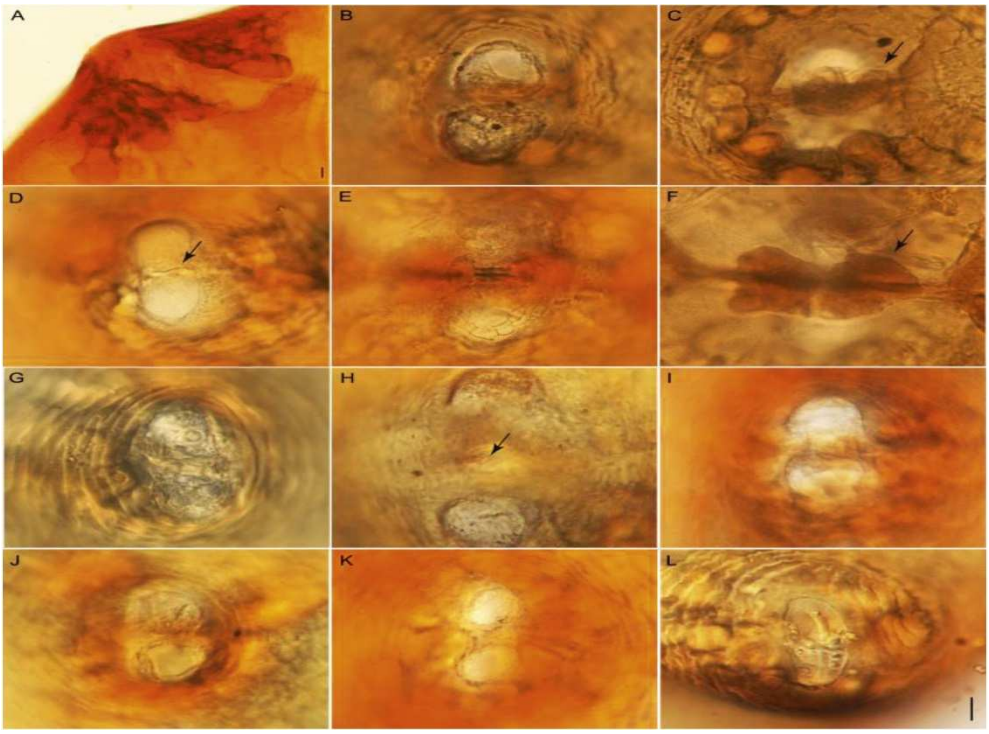


Figure 1.7. Vulval plates of species from the Avenae group. A, Lateral view of vulval cone with bullae; B, *Heterodera filipjevi*, anterior view; C, *H. filipjevi*, underbridge level view (underbridge is indicated by arrow); D, *H. avenae*, anterior view (vulval slit is indicated by arrow); E, *H. hordecalis*, anterior view; F, *H. hordecalis* (underbridge with pronounced thickenings is indicated by arrow); G, *H. aucklandica*, anterior view; H, *H. latipons*, anterior view (vulval slit is indicated by arrow); I, *H. mani*, anterior view; J, *H. sturhani*, anterior view; K, *H. pratensis*, anterior view; L, *H. ustinovi*, anterior view. Scale bars = 10 µm (Smiley et al., 2017).

Molecular identification is based on Polymerase Chain Reaction (PCR). The molecular marker PCR-RFLPs are used for differentiating *H. avenae*, *H. filipjevi*, and *H. latipons* (Toumi et al., 2013; Yan et al., 2013; Waeyenberge and Viaene, 2015; Ferris et al., 1994; Subbotin et al., 2003; Subbotin et al., 1999). However, molecular techniques fail to distinguish pathotypes of *H. avenae* (Subbotin et al., 2001).

Management of *Heterodera avenae*

H. avenae cause serious crop damage in many countries; therefore, Integrated Nematode Management (INM) has received greatest attention (Smiley et al., 2017). Resistant wheat cultivars along with crop rotation, long fallow periods, and biological control agents can be used in some environments (Kerry and Crump, 1980; Fisher and Hancock, 1991).

Biological control

There are no successful commercial biological control agents for *H. avenae* (Smiley et al., 2017). In vitro and greenhouse trials show that " nematophageous fungi " such as *Pochonia chlamydosporia* (Kerry and Crump, 1977), *Nematophthora gynophila* (Kerry and Crump, 1980), and *Paecilomyces lilacinus (*Vats et al., 2004; Khan et al., 2006) are antagonistic to *H. avenae.*

Chemical control

Nematicides as seed treatments, soil treatments, and foliar applications have resulted in effective control of *H. avenae* in Australia and India (Rivoal and Nicol, 2009). No nematicides are currently registered for controlling *H. avenae* on small grains in the U.S (Smiley et al., 2017). In Australia, Temik (Aldicarb) at 9 kg/ha led to complete elimination of cyst reproduction of *H. avenae* on wheat and barley (Brown, 1973). In Saudi Arabia, Fenamiphos and Urea reduced the number of nematode cysts per root in sandy soils (Al-Hazmi and Dawabah, 2014). Carbofuran was also reported to be effective in reducing the population of *H. avenae* on wheat (Kaushal and Seshadri, 1989). Methylene bisthiocyanate (MBT) and MTB+ thiamethoxam (MTT), four common pesticides fipronil+ chloropyrifos (FIC), ememectine benzoate, and the fungicide iprodione were effective in reducing the cysts

and eggs of *H. avenae* on wheat compared to untreated control, but MTT was most effective (Cui et al., 2016). Seed treatment with the fungicide Thiabendazole caused the highest reduction in cyst numbers on both susceptible and moderately resistant wheat genotypes compared to the controls (Dababat et al., 2014).

Resistance

One of the most effective control methods against *H. avenae* is the use of resistant cultivars (Rivoal and Nicol, 2009). Several mechanisms in plants can function to stop reproduction of the nematode, including toxic root exudates, lack of nematode larval attractant or egg hatching stimulation in the root exudates, a barrier to penetration or failure of nematode to develop within plant tissue (Webster, 1969). Resistance to *H. avenae* results in few or no cysts (Empson and Gair, 1982). The development of resistant cultivars may involve 10 to 12 years of research and screening of several breeding lines (Hoard and Cotton, 1978). Six resistant genes (*Cre*-genes) were reported to stop the reproduction of *H. avenae* on wheat. Cre*1* is effective against *H. avenae*, but only the wheat cultivar WB Rockland is carrying this gene in the PNW (Smiley et al., 2017).

Hatching of *Heterodera avenae*

Some cyst-forming nematodes are characterized by dormancy in which the development of unhatched J2 is arrested. Dormancy is divided into quiescence and diapause. Quiescence is broken when favorable conditions return, while diapause, especially the diapause programmed with life cycle (obligatory) requires specific conditions to be broken for development to resume (Perry, 2002). The hatching process is generally divided into three steps: changes in eggshell permeability, activation of the J2, and hatching of the J2 from the

egg (Smiley et al., 2017). There are two different hatching behaviors according to the geographical origin of populations: winter activity in Mediterranean climate regions, and spring activity in the Northern climate regions (Evans and Perry, 1976). In the Mediterranean climate, hatching mainly occurs during winter, while the northern population hatches in the spring. English populations of *H. avenae* hatched at 10℃ and 15℃ after storage for 8 weeks at 2℃ (Williams and Beane, 1979). In a comparison study between Egyptian and German populations of *H. avenae*, Baklawa et al. (2017) found the highest emergence of the J2 of the Egyptian population was observed between 10℃ and 15℃, compared to the German population which was at 5℃.

Thesis objectives

A fundamental understanding of hatching and development is important in studying the cereal cyst nematode, *H. avenae*. Past research has shown that the dormant second stage J2 of *H. avenae* require periods of low temperatures to hatch, but the optimum duration of low temperature exposure is no clear. Likewise, formation of white females on roots is important in evaluating the reproduction of *H. avenae*, but the optimum time of evaluation following inoculation has not been identified. The objectives of this thesis were to: (i) induce hatching of J2 of *H. avenae* by storing dormant eggs and encysted eggs at extended low temperature; and (ii) inoculate these ready to hatch eggs and encysted eggs on wheat and determine the time to form the white females on wheat roots.

Literature Cited

Al-Hazmi, A.S., Al-Yahya F.A., Abdul-Razig, A.T. (1999). Damage and Reproduction Potentials of *Heterodera avenae* on Wheat under Outdoor Conditions. Journal of Nematology 31: 662–666.

Al-Hazmi, A. S., and Dawabah, A. A. M. (2014). Effect of Urea and Certain NPK Fertilizers on the Cereal Cyst Nematode (*Heterodera avenae*) on Wheat. Saudi Journal of Biological Sciences 21: 191–196. Retrieved from http://doi.org/10.1016/j.sjbs.2013.10.002

Ali, M.A., Azeem, F., Li, H., and Bohlmann, H. (2017). Smart Parasitic Nematodes Use Multifaceted Strategies to Parasitize Plants. Frontiers Plant Science. 8:1699. Retrieved from https://doi.org/10.3389/fpls.2017.01699

Al-Yahya, F. A., Alderfasi, A. A., Al-Hazmi, A. S., Ibrahim, A.A.M. and Abdul-Razig, A. T. (1998). Effect of the Cereal Cyst Nematode on Growth and Physiological Aspects of Wheat under Field Conditions. Pakistan Journal of Nematology 16: 55-62.

Andersen, S. and Andersen, K. (1982). Suggestions for Determination and Terminology of Pathotypes and Genes for Resistance in Cyst-forming Nematodes, Especially *Heterodera avenae*. EPPO Bulletin 12, 379-386.

Baklawa, M., Bjorn, N., and Massoud, S. (2017). Influence of Temperature and Storage Conditions on the Hatching Behavior of Cereal Cyst Nematodes (*Heterodera avenae* Wollenweber) from Egypt. Journal of Plant Disease and Protection 124: 213-225.

Baldwin, J. G., and Mundo-Ocampo, M. (1991). Heteroderinae, Cyst- and non-Cyst-forming Nematodes. Pages 275-362 In: Manual of Agricultural Nematology. W. R. Nickle, ed. Marcel Dekker, New York.

Brown, R.H. (1973). Chemical Control of the Cereal Cyst Nematode (*Heterodera avenae*) - A Comparison of Methods and Rates of Application of Two Systemic Nematicides. Australian Journal of Experimental Agriculture 13: 64.

Brown, R. H. (1984). The Ecology and Control of Cereal Cyst Nematode (*Heterodera avenae)* in Southern Australia. Journal of Nematology 16: 216-222.

Chen, C., Liu, S., Liu, Q., Niu, J., Liu, P., Zhao, J. (2015). An Annexin-Like Protein from the Cereal Cyst Nematode *Heterodera avenae* Suppresses Plant Defense. PloS One 10. Retrieved from https://doi.org/10.1371/journal.pone.0122256

Cui, J. K., Peng, D., Huang, W., Peng, H., Lv, Y., Kong, L., li, H., Luo, S., and Wang, Y. (2016). Efficacy Evaluation of Seed-Coating Compounds Against Cereal Cyst Nematodes and Root Lesion Nematodes on Wheat. Plant Disease 101: 1094.

Dababat, A. A., Pariyar, S. R., Nicol, J. M., Erginbas-Orakci, G., Wartin, C., Klix, M., Bolat, N., Braun, H. J., and Sikora, R. A. (2014). Influence of Fungicide Seed Treatment on the Integrated Control of *Heterodera filipjevi* on Six Wheat Germplasm with Different Levels of Genetic Resistance under Controlled Conditions. Nematropica 44: 25-30.

Empson, D.W., and Gair, R. (1982). Nematodes (eelworms). Cereal Pests. Mint. Agriculture. Fish and Food. London. HMSO, 2nd ed, 92-97.

Evans, A. A. F. and Perry, R. N. (1976). Survival Strategies in Nematodes. The Organization of Nematodes 383-424.

Fisher, J.M., and Hancock, T.W. (1991). Population Dynamics of *Heterodera avenae* Woll in South Australia. Australian Journal of Agricultural Research 42: 53-68.

Ferris, V.R., Ferris, J.M., Faghini J., and Ireholm, A. (1994). Comparisons of Isolates of *Heterodera avenae* Using 2-D PAGE Protein Patterns and Ribosomal DNA. Journal of Nematology 26: 144–151.

Hafez, S. L., and Golden, A.M. (1985). First Report of Oat Cyst Nematodes (*Heterodera avenae*) on Barley in Idaho. Plant Disease 69:360.

Handoo, Z. A. (2002). Key and Compendium to Species of the *Heterodera avenae* Group (Nematode: Heteroderidae). Journal of Nematology 34: 250-262.

Howard, H.W., and Cotton, J. (1978). Nematode Resistant Crop Plants. In. Plant Nematology 3rd.ed. Southey, J.F. 313-325.

Ibrahim, I. K. A. and Handoo, Z. A. (2007). A Survey of Cyst Nematodes (*Heterodera* sp.) in Northern Egypt. Pakistan Journal of Nematology 25: 335-337.

Ibrahim, I. K. A., Rezk, M. A., and Ibrahim, A. A. M. (1986). Occurrence of the Cyst Nematodes *Heterodera avenae*, *Heterodera daverti* and *Heterodera rosii* in Northern Egypt. Journal of Nematology 18: 614-614.

Ibrahim, A.A., Al-Hazmi, A.S., Al-Yahya, F.A, and Alderfasi, A.A. (1999). Damage Potential and Reproduction of *Heterodera avenae* on Wheat and Barley under Saudi Field Conditions. Nematology 6: 625–630.

Jensen, H. J., Eshtiaghi, H., Koepsell, P.A., and Goetze, N. (1975). The Oat Cyst Nematode, *Heterodera avenae*, Occurs on Oats in Oregon. Plant Disease Reporter 59: 1–3.

Kaushal, K.K., and Seshadri, A.R. (1989). Effectiveness of Chemicals Against *Heterodera avena*e Woll on Wheat. Indian Journal of Nematology 19: 41-43.

Kerry, B. R. and Crump, D. H. (1977). Observations on Fungal Parasites of Females and Eggs of the Cereal Cyst Nematode, *Heterodera avenae*, and other Cyst Nematodes. Nematologica 23: 193-201.

Kerry, B. R. and Crump, D. H. (1980). 2 Fungi Parasitic on Females of Cyst-Nematodes (*Heterodera* spp). Transactions of the British Mycological Society 74: 119-125.

Khan, R. M., Agarwal, V. and Mathur, B. N. (1990). Chemical Control of Simultaneous Cereal Cyst Nematode and Termite Infestations in Wheat in Rajasthan, India, RACHIS (ICARDA) Barley and Wheat Newsletter 9: 9-11

Khan, A., Williams, K. L. and Nevalainen, H. K. M. (2006). Control of Plant-Parasitic Nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* in Pot Trials. Biocontrol 51: 643-658.

Liu, J., Peng, H., Cui, J., Huang, W., Kong, L., Clarke, J. L. (2016). Molecular Characterization of a Novel Effector Expansin-Like Protein from *Heterodera avenae* that Induces Cell Death in *Nicotiana benthamiana*. Scientific Reports 6:35677. doi: 10.1038/srep35677

Mactode Publications, Bugwood. Org. (2011). Retrieved from: https://www.forestryimages.org/browse/detail.cfm?imgnum=5441817

Mulvey, R.H. (1972). Identification *of Heterodera* Cysts by Terminal and Cone Top Structures. Canadian Journal of Zoology 50: 1277-1292.

National Agricultural Statistics Services. (2020, September 30). United States Department of Agriculture • Washington, *DC 20250* [Press release]. Retrieved from: https://www.nass.usda.gov/Statistics_by_State/Idaho/Publications/Crops_Press_Releases/2020/SG09_1.pdf

Patel, N., Hamamouch, N., Li, C. Y., Hewezi, T., Hussey, R. S., Baum, T. J. (2010). A Nematode Effector Protein Similar to Annexins in Host Plants. Journal of Experimental Botany 61, 235–248. doi: 10.1093/jxb/erp293

Peng, D.L., Nicol, J., Li H.M., Hou, S.G., Li H.X., Chen,S.L., Ma, P., Li H.L., and Riley, I.T. (2009). Current Knowledge of Cereal Cyst Nematode (*Heterodera avenae*) on Wheat in China. In: Riley, I.T., Nicol, J.M. et Dababat, A.A.(Eds). Cereal Cyst Nematodes: Status, Research and Outlook. Ankara, Turkey, CIMMYT. pp. 29-34.

Perry, R. N. (2002). Hatching. Pages 147-169 In: The Biology of Nematodes. D. L. Lee, ed. Taylor and Francis, London.

Rammah, A. (1994). Cereal Cyst Nematode (*Heterodera avenae*) in Morocco. Fifth Arab Congress of Plant Protection, Fez, Morocco, 27th November- 2nd December. Pp.199.

Rivoal, R., and Cook, R. (1993). Nematode Pests of Cereals. In: Evans, K., Trudgill, D.L. et Webster, J.M. (Eds). Plant Parasitic Nematodes in Temperate Agriculture. Wallingford, UK, CABI Publishing, pp. 259–303.

Rivoal, R. and Nicole, J. M. (2009). Past Research on the Cereal Cyst Nematode Complex and Future Needs, Addis Ababa, Ethiopia, International Maize and Wheat Improvement Centre (CIMMYT).

Riley, I.T., and McKay, A.C. (2009). Cereal Cyst Nematode in Australia: Biography of a Biological Invader. In: Riley, I.T., Nicol, J.M. et Dababat, A.A. (Eds.). Cereal Cyst Nematodes: Status, Research, and Outlook. CIMMYT, Ankara, Turkey. pp. 23–28.

Sampedro, J., and Cosgrove, D. J. (2005). The Expansin Superfamily. Genome Biology 6:242. doi: 10.1186/gb-2005-6-12-242

Sawarup, G., and Sosa-Moss, C. (1990). Nematode Parasites of Cereals. Pp. 109–136 in M. Luc, R. A. Sikora, and J. Bridge, eds. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Wallingford, UK: CAB International.

Simon, A. (1980). A Plant Assay of Soil to Assess Potential Damage to Wheat by *Heterodera avenae.* Plant Disease 64: 917–919.

Singh, A.K., Sharma, A.K., and Shoran, J. (2009). *Heterodera avenae* and Its Management on Wheat in India. In: Riley, I.T., Nicol, J.M. et Dadabat, A.A. (Eds). Cereal cyst Nematodes: Status, Research, and Outlook, CIMMYT: Ankara, Turkey. pp 149–153.

Sikora, R. A. (1988). Plant Parasitic Nematodes of Wheat and Barley in Temperate and Temperate Semi-Arid Regions – A comparative Analysis. In: Saxena, M. C., Sikora, R. A. & Srivastava, J. P. (eds.) Nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions. Publication, ICARDA, International Center for Agricultural Research in the Dry Areas, Syria.

Smiley, R.W. (2016). Cereal Cyst Nematodes Biology and Management in Pacific Northwest Wheat, Barley, and Oat. A Pacific Northwest Extension Publication. PNW 620.

Smiley, R. W., Ingham, R.E., Uddin, W., and Cook, G.H. (1994). Crop Sequences for Winter Wheat in Soil Infested with Cereal Cyst Nematode and Fungal Pathogens. Plant Disease 78: 1142–1149.

Smiley, R. W., Whittaker, R. G., Gourlie, J. A., Easley, S. A., and Ingham, R. E. (2005). Plant-Parasitic Nematodes Associated with Reduced Wheat Yield in Oregon*:* *Heterodera avenae*. Journal of Nematology 37: 297-307.

Smiley, R. W., and Nicol, J. M. (2009). Nematodes which Challenge Global Wheat Production. Pages 171-187 in: Wheat Science and Trade. B. F. Carver, ed. Wiley-Blackwell, Ames, IA.

Smiley, R.W., and Yan, G. (2010). Cereal Cyst Nematodes. A Pacific Northwest Extension Publication. Oregon State University. University of Idaho. Washington State University. PNW620. October 2010.

Smiley, R.W., Yan, G., and Pinkerton, J.N. (2011). Resistance of Wheat, Barley, and Oat to *Heterodera avenae.* in the Pacific Northwest, USA. Nematology 5: 539-552.

Smiley, R.W., Dababat, A.A., Iqbal, S., Jones, M.G., Maafi, Z.T., Peng, D., Subbotin, S.A., and Waeyenberge, L. (2017). Cereal Cyst Nematodes: A Complex and Destructive Group of *Heterodera* Species. Plant Disease 101: 1692- 1720.

Sturahan, D. (1982). Distribution of Cereal and Grass Cyst Nematodes in West Germany. Bulletin OEPP 12: 321-324.

Subbotin, S. A., Waeyenberge, L., Molokanova, I. A., and Moens, M. (1999). Identification of Species from the *Heterodera avenae* Group by Morphometrics and Ribosomal DNA RFLPs. Nematology 1: 195-207.

Subbotin, S. A., Rumpenhorst, H. J., Sturhan, D., and Moens, M. (2001). Description of the Australian Cereal Cyst Nematode *Heterodera australis* sp. n. (Tylenchida: Heteroderidae). Russian Journal of Nematology 10: 139-148.

Subbotin, S. A., Sturhan, D., Rumpenhorst, H. J. and Moens, M. (2003). Molecular and Morphological Characterization of the *Heterodera avenae* species Complex (Tylenchida: Heteroderidae). Nematology 5: 515-538.

Toumi, F., Waeyenberge, L., Viaene, N., Dababat, A., Nicol, J. N., Ogbonnaya, F., and Moens, M. (2013). Development of Two Species-Specific Primer Sets to Detect the Cereal Cyst Nematodes *Heterodera avenae* and *H. filipjevi*. European Journal of Plant Pathology 136: 613–624.

Vanstone, V. (2008). Root Disease under Intensive Cereal Systems. Department of Agriculture and Food, Western Australia, Bulletin 4732.

Vats, R., Kanwar, R. S. and Bajaj, H. K. (2004). Biology of *Seinura paratenuicaudata Geraert*. Nematologia Mediterranean 32: 117–121.

Williams, T. D., and Beane, J. (1979). Temperature and Root Exudates on the Cereal Cyst Nematode *Heterodera avenae*. Nematologica 25: 397-405.

Williams, T. D., and Beane, J. (1982). Variations in cereal Yield Losses Associated with *Heterodera avenae* in England and Wales. EPPO Bulletin 12: 485–490.

Waeyenberge, L., and Viaene, N. (2015). Molecular Identification of Cereal Cyst Nematodes: Status, Prospects, and Recommendations. In: Dababat, A.A., Muminjanov, H et al Smiley, R.W. (Eds). Nematodes of Small Grain Cereals: Status and Research. FAO, Ankara, Turkey. pp. 239–250.

Webster, J.M. (1969). The Host-Parasitic Relationships of Plant Parasitic Nematodes. Advanced Parasitology 7: 1-40.

Yan, G.P., Smiley, R.W., Okubara, P.A., and Skantar, A.M. (2013). Species-Specific PCR Assays for Differentiating *H. filipjevi and H. avenae*. Plant Disease 97: 1611–1619.

Yang, D., Chen, C., Liu, Q., and Jian, H. (2017). Comparative Analysis of Pre- and Post-Parasitic Transcriptomes and Mining Pioneer Effectors of *Heterodera avenae.* Cell Bioscience 7: 11-35.

Youssif, G.M. (1987). Effect of *Heterodera avenae* Populations in Infested Field Patches of Wheat Cultivation on Plant Growth and Yield. Paper presented at the 10th Symposium on the Biological Aspects of Saudi Arabia, Saudi Biological Society, Jeddah, Saudi Arabia. 20-24 April 1987.

Chapter 2: Effect of Temperature on Inducing Hatching of J2 and Development of White Females of *Heterodera avenae*.

Abstract

Hatching of second-stage juveniles (J2) of *H. avenae* is a complex biological process that varies with different geographical and climatic regions. Three tests were carried out to study the effect of temperature on hatching of J2 and the timing of development of white females of *H. avenae* on wheat. Dormant eggs and encysted eggs were stored at 4℃, 20℃, or at fluctuation temperatures of 4℃ and 20℃ before they were inoculated on wheat under greenhouse conditions. The overall hatching percentages were very low and did not exceed 11.0% in all experiments. Higher levels of hatching of J2 were induced by exposure of dormant eggs to 4℃ compared to 20℃. No hatched J2 or developed white females occurred when dormant encysted eggs were stored at 20□ and at fluctuation temperatures of 4 □ and 20℃. Generally, developed white females are anticipated 9 weeks after emergence of seedlings based on the two experiments. The current studies indicate that the dormant stage of J2 as dormant eggs or encysted eggs can be induced to hatch by extended exposure to 4℃. The resulting inoculum can be used for research on *H. avenae* biology and management. Knowing the time of hatching J2 and the development of white females on wheat roots is useful for designing experiments the efficacy of control options such as nematicides, and for enhancing the accurate evaluation *H. avenae* reproduction.

Introduction

The cereal cyst nematode, *Heterodera avenae* Woll is considered an important economic pest of wheat in Idaho (Hafez and Golden, 1985; Smiley, 2016). Hatching behavior

and the subsequent development are important biological aspects of *Heterodera avenae*. The hatching process of eggs of *H. avenae* is generally divided into three steps: changes in eggshell permeability, activation of the J2, and hatching of the J2 from the egg (Perry, 2002). There are two different hatching behaviors according to the geographical origin of populations: winter activity in Mediterranean climate regions, and spring activity in northern climate regions (Evans and Perry, 1976; Perry, 2011). Rivoal, (1978) found that in the Mediterranean climate hatching occurred mainly during winter, while in the northern climate hatching occurred in the spring. Hatching of the Canadian population of *H. avenae* was stimulated by incubation at low temperatures between 0℃ and 7℃ (Fushtey and Johnson, 1966). English populations of *H. avenae* hatched at 10℃ and 15℃ after storage for 8 weeks at 2℃ (Williams and Beane, 1979). At constant temperatures, the northern French population of *H. avenae* (Fr4) did not hatch at 20 and 25℃ and only hatched at 3℃ and 7℃. In contrast, the Algerian population hatched at the higher temperatures of 20 and 25℃ (Mokabli et al, 2001). In a comparison between Egyptian and German populations of *H. avenae*, Baklawa et al. (2017) found the highest emergence of J2 of the Egyptian population was observed between 10℃ and 15℃, whereas the highest emergence of J2 of the German population was observed at 5℃.

Studying *H. avenae* requires breaking the dormancy of eggs because current-season eggs and encysted eggs enter a dormant stage after natural hatching cycles (Rivoal, 1978). *H. avenae* exhibit obligate diapause which is programmed into its life cycle (Perry, 2002). In eastern Oregon, Guiping et al. (2013) concluded that collecting juveniles for use as inoculum would be very difficult after only 3.1%-3.2% hatching occurred in two populations of *H. avenae.*

Culturing *H. avenae* poses a challenge because of the dormancy of J2 and the slow reproduction. Moreover, the hatching window for *H. avenae* is short and varies between seasons (Smiley and Marshall, 2016). Collecting soils from infested fields with *H. avenae* after wheat harvest in late summer is the main source of inoculum to carry out research on *H. avenae* in the Pacific Northwest (Smiley et al., 2011). Determining the time of hatching J2 and appearance of the developed white females of *H. avenae* on wheat roots are important for evaluating the efficacy of management practices under controlled conditions. Developing a system to reliably break the dormancy and induce hatching of J2 are essential toward these research purposes. Two experiments consisted of three hatching tests were conducted to determine the effect of cold temperature on inducing hatching and determining the subsequent development of *H. avenae*.

Materials and Methods

First Experiment:

Hatching Test 1. Storing dormant eggs for two-, four-, and eight-week period at 4℃ or 20℃.

Nematode identification. Eight random cysts were picked from infested soil collected from eastern Idaho and shipped for molecular identification to the Mycology and Nematology Genetic Diversity and Biology Laboratory in Beltsville, MD. They were confirmed as *H. avenae* based on PCR-RFLPs. Morphological identification was made at the Nematology lab in Parma Research and Extension Center Parma, ID by using the terminal cyst cone structures and vulval slit (Mulvey, 1972) to further confirm the inoculum source was a pure of *H. avenae.*

Nematode inoculum preparation. Naturally infested soils were collected during the summer of 2018 from commercial fields near St. Anthony, Idaho. Cysts were collected and identified as originating from a current-season infestation, indicating that cysts had not been exposed to winter temperature cycles. Cysts were extracted by the sugar flotation technique (Jenkins, 1964) and crushed by hand to release the eggs. The resulting egg suspension was passed through 70 mesh sieves (212 µm) to remove the crushed cysts and debris and then passed through 230 mesh sieves (63µm) to remove any J2. Eggs were collected in a beaker. Pots (500 cm^3, 7.6 cm x 10.5 cm) were filled with sterilized sand and clay 50% each. Small holes were made in soil to receive the eggs and 5 eggs/cm^3 soil (2500 eggs/pot) were inoculated by syringe. All pots were stored at constant temperature of 4℃ or 20℃ for two, four, and eight weeks starting from 01 Aug. 2018.

Experimental design. Twelve pots were stored for each of durations (2, 4, or 8 weeks) at 4℃ in a refrigerator, and 12 pots were stored in a room at 20℃ for the same period. A thermometer was placed in each group of pots to monitor temperature. After each storage period, all pots moved to the greenhouse and were arranged in a complete randomized design (CRD). Six fungicide-treated seeds of wheat cv ‘Alturas’ provided by the Parma Research and Extension Center were planted in each pot and thinned to two per pot after germination which happened in three days. The greenhouse was equipped with Enviro STEP technology (WADSWORTH control system) with two analog input channels for connecting fluorescent night light, RH, precipitation, and temperature sensors and was set for 20℃ and 100% RH. Pots were watered as needed (daily monitoring wet/dry conditions).

Recording hatched J2 and developed white females. Four pots of each treatment were assessed for hatched J2 and developed white females 4, 9, and 13 weeks after emergence

of seedlings. J2 and white females were extracted from the soil and root with the earlier mentioned technique. Briefly, the sugar flotation technique involves washing soils and roots thoroughly and then pouring the water through a series of different-sized sieves. Cysts are collected in cups at a sieve size of 70 mesh (212µm) and egg-J2 at a sieve size of 400 mesh (38µm). The collected nematode life stages are then concentrated with the centrifuge (International Centrifuge, model SBV, Boston, MASS) at a speed of 1500 rpm for five minutes and then at speed of 2000 rpm for one minute with sugar concentration of 1587.5 grams of sugar diluted in 1 liter of water for cysts and 907.2 grams of sugar diluted in 1 liter of water for J2. Hatched J2 were counted with the aid of a Leica light microscope (Wetzlar, Germany)., and the developed white females were counted under Leica dissecting microscope (Wetzlar, Germany). Hatching percentage was calculated based on numbers of hatched J2 and developed white females divided by the total eggs inoculated into each pot (Guiping et al., 2013).

Hatching Test 2. Storing dormant encysted eggs for two-, four-, and eight-week period at 4℃ or 20℃.

Inoculum preparation, hatching recording, and experimental design. Ten non crushed current-season cysts, extracted from St. Anthony infested soil, were inoculated into the same soil and pot size as in in hatching test 1. The initial inoculum level in each replicate (pot) was calculated by crushing two cysts and calculating the average number of eggs per cyst = 250 ±10 eggs/cyst. All pots were stored for two, four, and eight weeks at 4℃ or 20℃ starting from 01 Aug. 2018. Twelve pots were stored for each duration and temperature combination. After each storage period, pots were moved to the greenhouse and six seeds of wheat cv ‘Alturas’ were planted in each pot and thinned to two plants per pot after

germination. Hatched J2 and developed white females were counted as described in hatching test 1, and hatching percentage was calculated as described in hatching test 1 as well.

Hatching Test 3. Storing dormant eggs and encysted eggs for eight-weeks at fluctuating temperatures of 4℃ and 20℃.

Inoculum preparation and experimental design. This test inoculating pots with either dormant eggs or dormant encysted eggs at the same inoculum level as described in hatching tests 1 and 2, respectively. Pots were stored at fluctuating temperatures (12hr at 4 ℃ and 12hr at 20℃) for eight weeks. Pots were maintained with adequate moisture, and a thermometer was placed in each group. After eight weeks, the pots were moved to the greenhouse, placed under conditions previously described and arranged in a complete randomized design (CRD). Six seeds of wheat cv 'Alturas' were planted in each pot on 01 Oct. 2018 and thinned to two per pot after germination. Hatched J2 and developed white females were counted per pot, and hatching percentage was calculated 4, 9, and 13 weeks after emergence of seedlings with the same extraction and counting procedure as described in hatching test 1.

Second Experiment:

Hatching tests 1, 2, and 3 were repeated between 2020-2021. Infested soil with *H. avenae* was collected in October 2020 from eastern Idaho and cysts were extracted in the nematology lab at Parma Research and Extension Center. Dormant eggs and encysted eggs were prepared and stored at the same temperatures and storage durations as described in the first experiment. Hatched J2 and developed white females were extracted and counted as

described in the first experiment. Hatching percentage was calculated as described in experiment 1.

Data analysis. Data were analyzed with SAS (Version 9.4, 2013), using General Linear Model (GLM). For hatching test 1, temperature, recording time, and the interaction between them were considered fixed effects and replication as random effect. Pots of each storage period were moved to the greenhouse and planted immediately with wheat. Because of discrepancy between the three storage periods and the microclimate variability in the greenhouse that could influence on data, each storage period was analyzed separately.

In hatching test 2, there was a complete lack of hatching and development of *H. avenae* regardless of storage duration or evaluation time when exposed to 20℃ soil temperature, resulting in an excessive number of zeros in the data. Additionally, there was no hatching or development when exposed to 4℃ soil temperatures except when evaluated 13 weeks after wheat seedling emergence. This resulted in the data violating the assumption of homogenous variances. Therefore, it was decided to conduct statistical analysis with storage period as fixed effect and replication as random effect only at the 13-week evaluation time. The General Linear Mixed Model (GLMMIX) was used to create interaction plots between the two storing temperatures and the three recording times for the developed white females.

There were also excessive zeros in the hatching test 3 data because none of the dormant encysted eggs hatched after 8 weeks of exposure to fluctuating temperatures of 4 and 20℃. Therefore, the data were analyzed to compare the three recording times.

Results

Hatching Test 1. Storing dormant eggs for two-, four-, and eight-week periods at 4℃ or 20℃.

First Experiment:

Data analysis showed significant effects of temperature (4℃ and 20℃) and recording times on the hatched J2, developed white females, and hatching percentage after two-, four-, and eight-week storing periods (Appendix 2). No significant differences were observed for the interaction between the two main effects on the number of hatched J2 and hatching percentage in the two-weeks storing period and the interaction was only significant in the number of developed white females (Appendix 2). The interactions were significant at the four- and eight-weeks storing periods for the number of developed white females, hatched J2, and hatching percentage (Appendix 2).

The highest number of hatched J2 and hatching percentage were found when dormant eggs were stored at 4℃ for eight-weeks storing period (Table 2.1). Although there was a high number of hatched J2 in all dormant eggs stored at 4℃, few white females developed. This phenomenon will be explained later in the discussion section.

No development of white females occurred 4 and 9 weeks after emergence of seedlings when dormant eggs were stored at 20℃ for the two-, four-, and eight-weeks storing periods (Figs 2.1, 2.2, and 2.3). Developed white females were found 9 weeks after emergence of seedlings when dormant eggs were stored at 4℃ for four and eight-weeks storing periods (Figs 2.2 and 2.3).

The accumulative effect of the two temperatures over the three- recording periods are shown in table (2.2). At the two-weeks storing period, significant differences were observed between the two temperatures in the mean of hatched J2 and hatching percentage and no significant difference was in the mean number of white females. Significant difference was only present between the two temperatures in the mean number of white females at the four-weeks storing period. The hatched J2, developed white females, and hatching percentage were significantly different between the two temperatures at the eight-weeks storing period. The highest numbers of hatched J2, developed white females, and hatching percentage were found at 4℃ for an eight-weeks storing period (Table 2.2).

Second Experiment:

Data analysis showed significant differences of the main effects of the two temperatures and the three recording times and the interaction between them on the developed white females and hatching percentage at eight weeks storing period (Appendix 3). Significant differences were present for the main effects and the interaction between them on the developed white females at four weeks storing period (Appendix 3). The main effect of temperature was not significant on J2 at any of the three storing periods (Appendix 3).

In accordance with the first experiment, no white females developed 4 weeks after emergence of seedlings when dormant eggs stored at 4℃ or 20℃ regardless of storage duration (Figs 2.4, 2.5, and 2.6). In contrast to the first experiment, white females were found 9 weeks after emergence of seedlings at storing temperature 4℃ for the two weeks storing period (Fig 2.4). Developed white females were found 9 weeks after emergence of seedlings when dormant eggs stored at 20℃ compared to the first experiment (Figs 2.5 and 2.6). The highest hatching percentage was recorded when dormant eggs were stored at 4℃ for eight

weeks and 13 weeks after emergence of seedlings (Table 2.3). Significant differences were present in the mean number of developed white females between the two storing temperatures of 4℃ and 20℃ in the four and eight storing periods over the three recording times (Table 2.4).

Hatching Test 2. Storing dormant encysted eggs for two-, four-, and eight-week periods at 4℃ or 20℃.

First Experiment:

No hatching and development of dormant encysted eggs occurred when they were stored at 20℃ for two-, four, and eight-weeks (Table 2.5). No hatching and development occurred until 13 weeks after wheat seedlings emergence when dormant encysted eggs were stored at 4℃ regardless of storage period (Table 2.5). Thirteen weeks after emergence of wheat seedlings there were no significant differences in the mean number of developed white females (Table 2.5). However, significant differences were present in the number of hatched J2 and hatching percentage (Table 2.5).

Second Experiment:

Results were similar to the experiment 1 and no hatching or development occurred when dormant encysted eggs were stored at 4℃ for 4 and 9 weeks after emergence of seedlings, regardless of storage duration (Table 2.6). Hatching and development occurred only13 weeks after emergence of seedlings when dormant encysted eggs were stored at 4℃ for two, four, and eight weeks (Table 2.6).

Hatching Test 3. Storing dormant eggs and encysted eggs for eight-weeks at fluctuating temperatures 4℃ and 20℃.

First Experiment:

No hatching or development occurred when dormant encysted eggs were stored at fluctuating temperature of 4℃ and 20℃ regardless of recording times (Table 2.7). In contrast, hatching and development of dormant eggs occurred at all three recording times under the same temperature conditions (Table 2.7). Developed white females were found 4, 9, and 13 weeks after emergence of seedlings (Table 2.7). No significant differences were observed in the mean numbers of hatched J2 and hatching percentage among the three recording times (Table 2.7).

Second Experiment:

Dormant encysted eggs did not hatch or develop to white females similar to the first experiment (Table 2.8). Dormant eggs exhibited the most hatching of J2 at the 4-week recording time, but the most white females developed at 9 and 13 weeks after wheat seedlings emergence.

Discussion

Hatching of J2 and the subsequent development of the cereal cyst nematode, *H. avenae* is greatly influenced by soil temperatures (Meagher, 1970; Rivoal, 1979). The findings from the current hatching tests support the findings of previous studies in that the low temperature induced hatching J2 of the northern ecotype of *H. avenae* (Fushtey and Johnson, 1966; Rivoal, 1983; Mokabil et al., 2001; Baklawa et al., 2017).

In this study, dormant eggs manually released from cysts by crushing were more influenced by low temperature of 4℃ than dormant encysted eggs in number of hatched J2 and development of white females. Moreover, storing dormant eggs at 4℃ resulted in more

developed white females compared to 20℃. The developed white female indicates a successful hatching and root penetration of J2. As mentioned in the results, few white females developed compared to the high number of hatched J2. This occurs because infectivity of hatched J2 of *H. avenae* is lost within three weeks after hatching (Davies and Fisher, 1976). The coincidence of hatched J2 and the newly sensitive germinated roots of wheat host is a determining factor for penetration and development of *H. avenae* (Smiley, 2016). The relatively small effect of low temperature exposure on dormant encysted eggs in our hatching tests might be attributed to the protective layer of the cyst. This layer is thick and is considered as barrier against adverse conditions such as the parasites that feed on eggs (Brown, 1984). Therefore, encysted eggs are less sensitive to cold exposure compared to un-encysted eggs. Releasing encysted J2 from the cyst could be another factor of few developed white females. Some hatched J2 failed to be released from the cysts and were found starved and unhealthy. A higher hatching rate and development of white females of *H. avenae* would be expected in infested soil if free eggs were detected.

Despite the fact that hatching of J2, and development of white females were not statistically compared among the three-storing period in dormant eggs, there was positive correlation between hatching percentage and duration of exposure to 4℃. Increased the duration of exposure to 4℃ increased hatching of J2 and development of white females. Extended overwintered conditions induced hatching J2 of the Canadian and English populations of *H. avenae* (Fushtey and Johnson, 1966; Williams and Beane, 1979; Perry, 2002). Smiley et al. (2011) used overwintered infested field soil as inoculum in their research on *H. avenae*. Overwintered eggs are described as eggs of *H. aveane* exposed to winter cold cycles (Rivoal, 1978; Smiley et al, 2011). Hatching cycle of *H. avenae* is synchronized by

certain temperatures and the coincidence of hatching cycles with emergence of wheat seedlings result in high infection (Baklawa, 2013). Damage of wheat happens when hatching cycles of *H. avenae* coincide with the emergence of roots (Smiley and Marshall, 2016). Knowing these temperatures allow wheat growers adjust planting dates and avoid the coincidence hatching peak of J2 of *H. avenae* and the sensitive stages of wheat seedlings.

None of dormant encysted eggs hatched or developed into white females when stored at 20℃ in this hatching study. Thus, these results agree with reports that the cysts of the northern French population of *H. avenae* did not hatch at 20℃ or 25℃ (Mokabli et al, 2001). No hatching or development of *H. avenae* occurred from dormant infested soil in greenhouse experiments at Parma Research and Extension Center before conducting this hatching study (Unpublished data).

Determining the time of hatching J2 and development of white females of *H. avenae* is important in application timing of nematicides (Smiley et al., 2011). The time of formation the white females on the roots should also be considered in evaluating the reproduction of *H. avenae* on wheat. White females turn brown at the end of growing season and result in error in differentiating between the new formed cysts and those remaining in soil in previous crops (Smiley and Marshall, 2016). In this study, the earliest white female developed was 4 weeks after emergence of seedlings when dormant eggs were stored at fluctuating temperatures of 4℃ and 20℃. However, developed white females were found 9 weeks after emergence of seedlings in the second greenhouse experiment of this hatching test. Generally, developed white females of *H. avenae* are anticipated to appear on wheat root 9 weeks after emergence of seedlings based on the findings of the current hatching tests. Accurate evaluation of the production of white females of *H. avenae* should not exceed 15 weeks after emergence of

seedlings if experiments are conducted in naturally infested soil. White females will turn brown by this time, and it is difficult to distinguish them from older cysts. Further investigation based on weekly recording of when developed white females appear on roots is required. In preliminary hatching tests, developed white females were only found 9 weeks after emergence of seedlings. Therefore, we recorded hatching and development after 4, 9, and 13 weeks instead of 4, 8, and 12 weeks.

Fluctuating temperatures between 4℃ and 20℃ for eight weeks induced hatching and accelerated development of white females from dormant eggs. Baklawa et al. (2017) found that storing encysted eggs of the German population of *H. avenae* at 5℃ for 8 or 12 weeks before incubation at 10℃ stimulated the hatching of J2. Moving pots between 4℃ and 20 ℃ every 12 hours created a thermal gradient between these two temperatures that might have accelerated hatching and development in the first experiment of this study. However, hatching behavior changed in the second experiment and no development of white females happened 4 weeks after emergence of seedlings. Exposing the cysts to different temperatures to simulate seasonal variations was previously reported to induce hatching of *H. avenae* (Rivoal, 1983; Mokabli et al., 2001; Baklawa et al., 2017).

The very low hatching percentages in these hatching tests agree with the low hatching percentages found in eastern Oregon. Guiping et al. (2013) reported only 3.1%-3.2% hatching occurred in two populations of *H. avenae.* In the current study, hatching percentages of all hatching tests were very low and did not exceed 11%.

In conclusion, the current hatching study indicates that storing at low temperature of 4℃ for extended periods allow the use of dormant eggs and encysted eggs as artificial inoculum. The time of hatching J2 and development of white females of *H. avenae* is critical

for the evaluation of the reproduction and the application timing of nematicides for this nematode. Early application of nematicides at the time of hatching J2 would be more effective than a rescue application after the appearance of white females on roots (Smiley et al., 2011). Error could happen in evaluating the reproduction of *H. avenae* if more white females turned brown and mixed with the previous crop cysts.

Literature Cited

Baklawa, M. (2013). Biology and Pathogenicity of Cereal Cyst Nematodes on Wheat in Ismailia, Egypt. (Publication No. am: 04.09. 2013)[Doctoral dissertation, Technical University Braunschweig]. Retrieved from: https://publikationsserver.tu-braunschweig.de/receive/dbbs_mods_00054150

Baklawa, M., Bjorn, N., and Massoud, S. (2017). Influence of Temperature and Storage Conditions on the Hatching Behavior of Cereal Cyst Nematodes (*Heterodera avenae* Wollenweber) from Egypt. Journal of Plant Disease and Protection 124: 213-225.

Brown, R. H. (1984). The Ecology and Control of Cereal Cyst Nematode (*Heterodera avenae)* in Southern Australia. Journal of Nematology, 16: 216-222.

Davies, K.A., and Fisher, J.M. (1976). Duration of Infectivity of Second Stage Larvae of *Heterodera avenae*. Nematologica 22;163-168.

Evans, A. A. F. and Perry, R. N. (1976). Survival Strategies in Nematodes. The Organization of Nematodes 383-424.

Fushtey, S. G. and Johnson, P. W. (1966). Biology of Oat Cyst Nematode *Heterodera avenae* in Canada. I. Effect of Temperature on Hatchability of Cysts and Emergence of Larvae Nematologica*,* 12**,** 313-320.

Guiping, Y., Smiley, R.W., and Gourlie, J. A. (2013). Hatching of *Heterodera filipjive* and *H. avenae* Populations in Oregon. Journal of Nematology 4: 328.

Hafez, S. L., and Golden, A.M. (1985). First Report of Oat Cyst Nematodes (*Heterodera avenae*) on Barley in Idaho. Plant Disease 69:360.

Jenkins, W.R. (1964). A Rapid Centrifugal Flotation Technique for Separating Nematodes from Soil. Plant Disease Report 48: 692.

Meagher, J.W. (1970). Seasonal Fluctuations in Numbers of Larvae of the Cereal Cyst Nematode (*Heterodera avenae*) and of *Pratylenchus minyus* and *Tylenchorhynchus brevidens* in Soil. Nematologica, 16, 333-347.

Mokabli, A., Valette, S., Gauthier, J. P. and Rivoal, R. (2001). Influence of Temperature on the Hatch of *Heterodera avenae* Woll. Populations from Algeria. Nematology*,* 3**,** 171-178.

Mulvey, R.H. (1972). Identification *of Heterodera* Cysts by Terminal and Cone Top Structures. Canadian Journal of Zoology 50: 1277-1292.

Perry, R.N. (2011). Understanding the Survival Strategies of the Nematodes. " CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 6.022 (2011): 1-5. Web.

Perry, R. N. (2002). Hatching. Pages 147-169 In: The Biology of Nematodes. D. L. Lee, ed. Taylor and Francis, London.

Rivoal, R. (1978). Biology of *Heterodera avenae* in France 1. Differences in Hatching and Development Cycles of 2 Races Fr1 and Fr4. Révue de Nématologie 1, 171-180.

Rivoal, R. (1979). Biology of *Heterodera avenae* in France 2. Comparative Study of Hatching Temperatures Between Fr1 and Fr4 Races. Révue de Nematologie, 2, 233-248.

Rivoal, R. (1983). Biology of *Heterodera avenae* Wollenweber in France. III. Evolution of Diapauses of Fr1 and Fr4 Races in Long-Term Experiments Influence of Temperature. Révue de Nématologie*,* 6**,** 157-164.

Smiley, R.W. (2016). Cereal Cyst Nematodes Biology and Management in Pacific Northwest Wheat, Barley, and Oat. A Pacific Northwest Extension Publication. PNW 620.

Smiley, R.W., Yan, G., and Pinkerton, J.N. (2011). Resistance of Wheat, Barley, and Oat to *Heterodera avenae* in the Pacific Northwest, USA. Nematology 13 (5), 539-552.

Smiley, R.W., and Marshall, J.M. (2016). Detection of Dual *Heterodera avenae* Resistance Plus Tolerance Traits in Spring Wheat. Plant Disease. 100: 1677-1685.

Williams, T. D., and Beane, J. (1979). Temperature and Root Exudates on the Cereal Cyst Nematode *Heterodera avenae*. Nematologica 25: 397-405.

Table 2.1. Hatched J2 and hatching % of the dormant eggs of *Heterodera avenae* that were stored for 2 to 8 weeks at 4℃ or 20℃ and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (first experiment).

Storing temperature	Time after wheat emergence (Weeks)	2-week storing period		4-week storing period		8-week storing period	
		Hatched J2/pot	Hatching%**	Hatched J2/pot	Hatching%	Hatched J2/pot	Hatching%
4℃	4	52.5*bc	2.8b	12.5c	0.5b	185.0c	7.4c
	9	82.5b	3.3b	72.5a	3.3a	225.0b	9.1b
	13	132.5a	5.6a	87.5a	3.7a	290.0a	11.8a
20℃	4	7.5d	0.3c	32.5b	1.3b	10.0e	0.4e
	9	15.0cd	0.6c	20.0bc	0.7b	7.5e	0.0e
	13	70.0b	2.9b	82.5a	3.3a	50.0d	2.0d

* Each number is a mean of four replications. Numbers followed by the same letter within each column of each storing period are not significantly different based on specified pair-wise comparisons at alpha = 0.05

** Hatching percentages were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (2500 eggs/pot) in each replication.

Table 2.2. Influence of the two temperatures 4℃ and 20℃ on hatched J2, developed white females (WF), and hatching % of the dormant eggs that were stored for 2 to 8 weeks over the three-recording times (first experiment).

Storing temperature	2-week storing period			4-week storing period			8-week storing period		
	Hatched J2/pot	WF/pot	Hatching %	Hatched J2/pot	WF/pot	Hatching %	Hatched J2/pot	WF/pot	Hatching %**
4℃	89.1*a	1.8a	3.8a	57.5a	3.3a	2.5a	233.3a	3.6a	9.4a
20℃	30.8b	1.2a	1.2b	45.0a	0.8b	1.7a	22.5b	0.6b	0.8b

* Each number is a mean of four replications averaged over three recording times. Numbers followed by the same letter in each column are not significantly different based on specified pair-wise comparisons at alpha = 0.05.

** Hatching percentages were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (2500 eggs/pot) in each replication.

Table 2.3. Hatched J2 and hatching % of the dormant eggs of *Heterodera avenae* that were stored for 2 to 8 weeks at 4℃ or 20℃ and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (second experiment).

Storing temperature	Time after wheat emergence (Weeks)	2-week storing period		4-week storing period		8-week storing period	
		Hatched J2/pot	Hatching%**	Hatched J2/pot	Hatching%	Hatched J2/pot	Hatching%
4℃	4	32.5*bc	1.3bc	60.0c	0.5c	12.5b	0.3c
	9	45.0bc	1.9bc	145.0a	5.8a	125.0a	9.1a
	13	87.5a	3.6a	122.5a	5.7a	120.0a	4.9b
20℃	4	20.0c	0.8c	32.5cd	1.3c	47.5ab	0.7c
	9	15.0c	0.6c	120.0ab	5.1a	20.0b	0.0c
	13	62.5ab	2.6ab	72.5c	3.1b	132.5a	5.3d

* Each number is a mean of four replications. Numbers followed by the same letter within each column of each storing period are not significantly different based on specified pair-wise comparisons at alpha = 0.05

** Hatching percentages were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (2500 eggs/pot) in each replication.

Table 2.4. Influence of the two temperatures of 4℃ and 20℃ on hatched J2, developed white females (WF), and hatching % of the dormant eggs of *Heterodera avenae* that were stored for 2 to 8 weeks over the three-recording times (second experiment).

Storing temperature	2-week storing period			4-week storing period			8-week storing period		
	Hatched J2/pot	WF/pot	Hatching %	Hatched J2/pot	WF/pot	Hatching %	Hatched J2/pot	WF/pot	Hatching %**
4℃	55.0*a	2.6a	2.2a	93.3a	5.5a	4.0a	85.8a	5.4a	4.7a
20℃	32.5b	1.1a	1.3b	75.0a	3.5b	3.1a	66.6a	1.1b	2.0b

* Each number is a mean of four replications averaged over three recording times. Numbers followed by the same letter in each column are not significantly different based on specified pair-wise comparisons at alpha = 0.05.

** Hatching percentages were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (2500 eggs/pot) in each replication.

Table 2.5. Hatched J2, developed white females, and hatching % of the dormant encysted eggs of *Heterodera avenae* that were stored for 2 to 8 weeks at 4℃ or 20℃ and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (first experiment).

Storing temperature	Time after wheat emergence (Weeks)	HatchedJ2/pot			Developed white females/pot			Hatching %**		
		2wks storing period	4wks storing period	8wks storing period	2wks storing period	4wks storing period	8wks storing period	2wks storing period	4wks storing period	8wks storing period
4℃	4	No hatching occurred			No development occurred			-	-	-
	9	No hatching occurred			No development occurred			-	-	-
	13	20.0*b	65.0a	20.0b	3.2a	2.2a	3.2a	0.9b	2.6a	0.9b
20℃	4	No hatching occurred			No development occurred			-	-	-
	9	No hatching occurred			No development occurred			-	-	-
	13	No hatching occurred			No development occurred			-	-	-

* Each number is a mean of four replications. Numbers followed by the same letter within each row of each storing period are not significantly different based on specified pair-wise comparisons at alpha = 0.05

** Hatching percentages were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (10 cysts/pot 250±10 eggs/cyst) in each replication.

Table 2.6. Hatched J2, developed white females, and hatching % of the dormant encysted eggs of *Heterodera avenae* that were stored for 2 to 8 weeks at 4℃ or 20℃ and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (second experiment).

Storing temperature	Time after wheat emergence (Weeks)	HatchedJ2/pot			Developed white females/pot			Hatching %**		
		2wks storing period	4wks storing period	8wks storing period	2wks storing period	4wks storing period	8wks storing period	2wks storing period	4wks storing period	8wks storing period
4℃	4	No hatching occurred			No development occurred			-	-	-
	9	No hatching occurred			No development occurred			-	-	-
	13	0.0*b	50.0a	7.5b	4.2a	3.2a	5.0a	0.1b	2.1a	0.1b
20℃	4	No hatching occurred			No development occurred			-	-	-
	9	No hatching occurred			No development occurred			-	-	-
	13	No hatching occurred			No development occurred			-	-	-

* Each number is a mean of four replications. Numbers followed by the same letter within each row of each storing period are not significantly different based on specified pair-wise comparisons at alpha = 0.05

** Hatching percentages were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (10 cysts/pot 250±10 eggs/cyst) in each replication.

Table 2.7. Hatched J2, developed white females, and hatching % of the dormant eggs and encysted eggs of *Heterodera avenae* that were stored at fluctuating temperatures of 4℃ and 20℃ for eight weeks and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (first experiment).

Storing temperature	HatchedJ2/pot			Developed white females/pot			Hatching%**		
	4 weeks	9 weeks	13 weeks	4 weeks	9 weeks	13 weeks	4 weeks	9 weeks	13 weeks
Dormant eggs at 4℃ and 20℃ for eight weeks	135.0*a	110.0a	172.5a	3.2b	7.2a	8.0a	5.3a	4.6a	7.2a
Dormant encysted eggs at 4℃ and 20℃ for eight weeks	No hatching occurred			No development occurred			-	-	-

*Each number is a mean of four replications. Means followed by same letter within each row are not significantly different based on specified pair-wise comparisons at alpha = 0.05.

** Hatching percentages% were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (2500 eggs/pot) and (10 cysts/pot 250±10 eggs/cyst) in each replication.

Table 2.8. Hatched J2, developed white females, and hatching % of the dormant eggs and encysted eggs of *Heterodera avenae* that were stored at fluctuating temperatures of 4℃ and 20℃ for eight weeks and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (second experiment).

Storing temperature	Hatched J2/pot			Developed white females/pot			Hatching%**		
	4 weeks	9 weeks	13 weeks	4 weeks	9 weeks	13 weeks	4 weeks	9 weeks	13 weeks
Dormant eggs at 4℃ and 20℃ for eight weeks	197.5*a	92.5b	95.0b	0.0c	5.7b	17.0a	7.9a	4.2b	4.5ab
Dormant encysted eggs at 4℃ and 20℃ for eight weeks	No hatching occurred			No development occurred			-	-	-

*Each number is a mean of four replication. Means followed by same letter within each row are not significantly different based on specified pair-wise comparisons at alpha = 0.05.

**** Hatching percentages% were calculated based on Guiping et al. (2013). Hatching % = number of hatched J2+developed white females/total number of eggs (2500 eggs/pot) and (10 cysts/pot 250±10 eggs/cyst) in each replication.

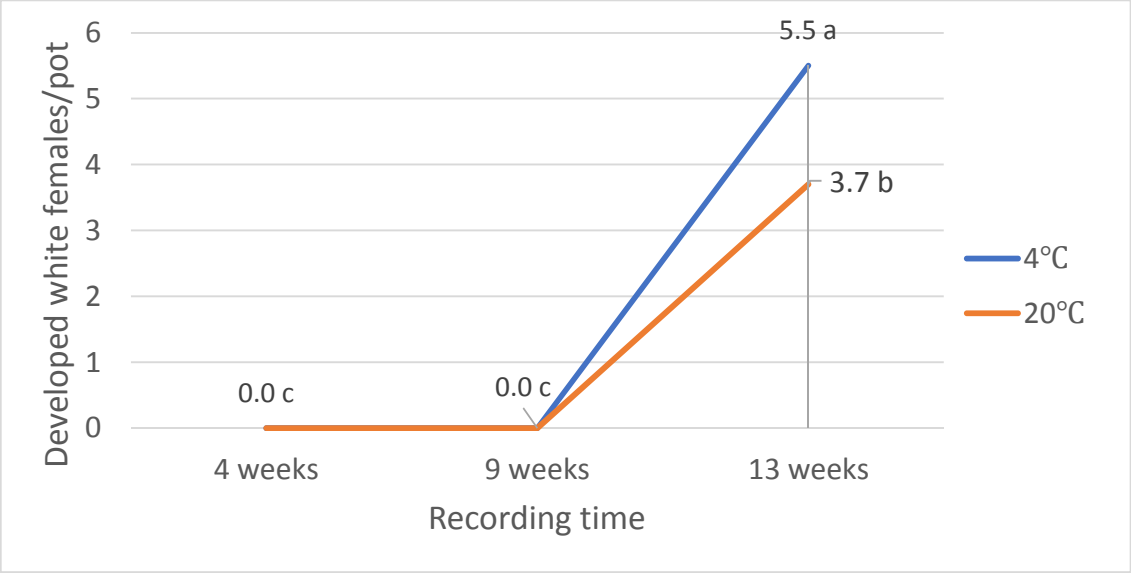


Figure 2.1. Interaction plot of the developed white females from dormant eggs that were stored for two weeks at 4°C or 20°C and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings. Each number is a mean of four replicates. Numbers followed by the same letter are not significantly different at α 0.05. The Y-axis represents the number of white females, and the X-axis represents the recording times (first experiment).

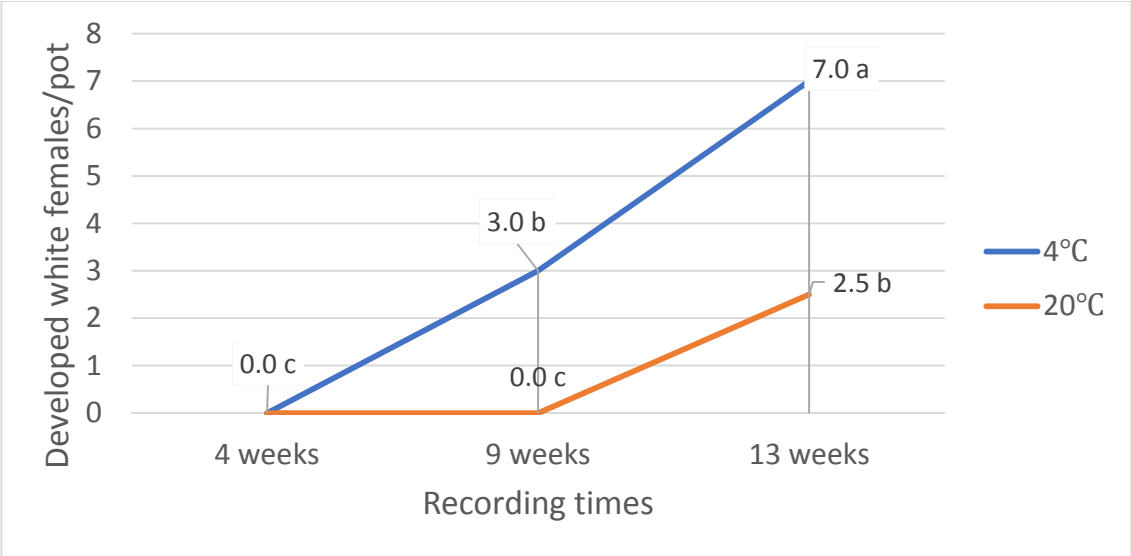


Figure 2.2. Interaction plot of the developed white females from dormant eggs that were stored for four weeks at 4°C or 20°C and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings. Each number is a mean of four replicates. Numbers followed by the same letter are not significantly different at α 0.05. The Y-axis represents the number of white females, and the X-axis represents the recording times (first experiment).

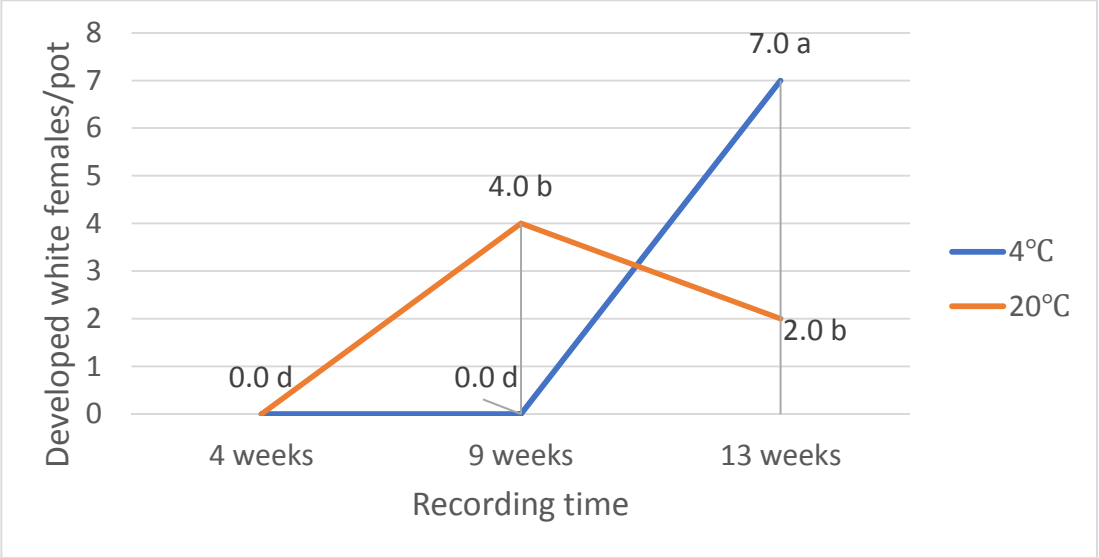


Figure 2.3. Interaction plot of the developed white females from dormant eggs which were stored for eight weeks at 4℃ or 20℃ and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings. Numbers followed by the same letter are not significantly different at α 0.05. The Y-axis represents the number of white females, and the X-axis represents the recording times (first experiment).

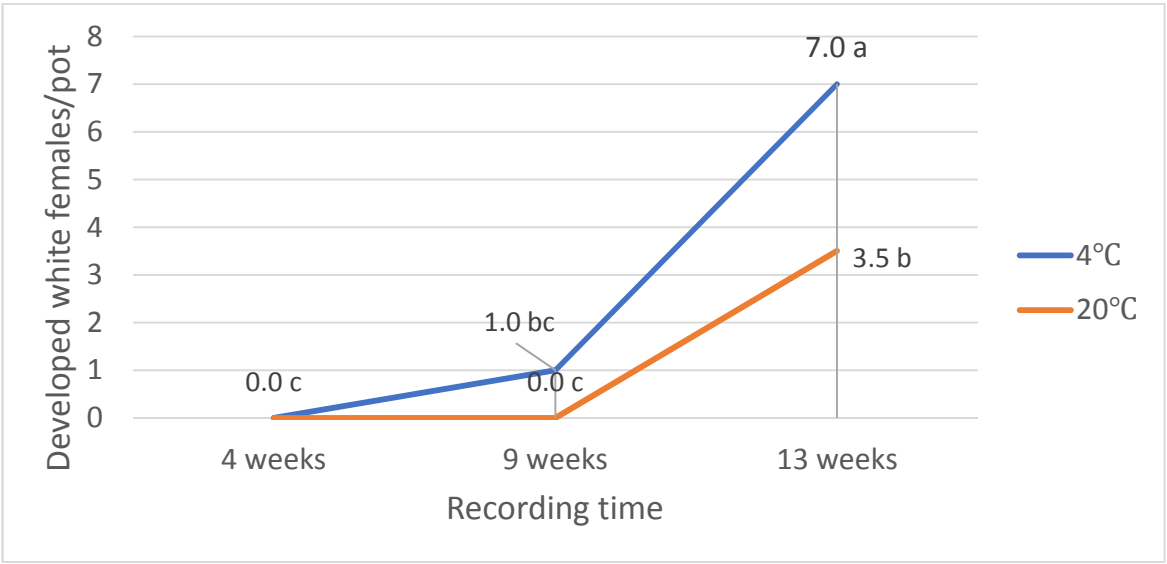


Figure 2.4. Interaction plot of the developed white females from dormant eggs which were stored for two weeks at 4℃ or 20℃ and then allowed to develop for4, 9, and 13 weeks after emergence of wheat seedlings. Numbers followed by the same letter are not significantly different at α 0.05. The Y-axis represents the number of white females, and the X-axis represents the recording times (second experiment).

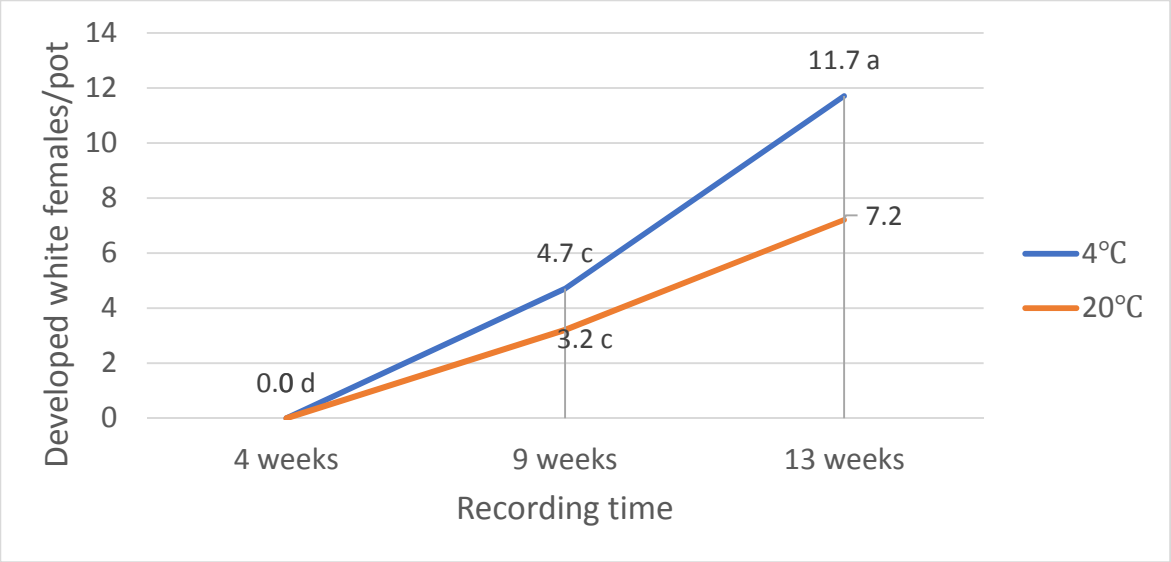


Figure 2.5. Interaction plot of the developed white females from dormant eggs which were stored for four weeks at 4°C or 20°C and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings. Numbers followed by the same letter are not significantly different at α 0.05. The Y-axis represents the number of white females, and the X-axis represents the recording times (second experiment).

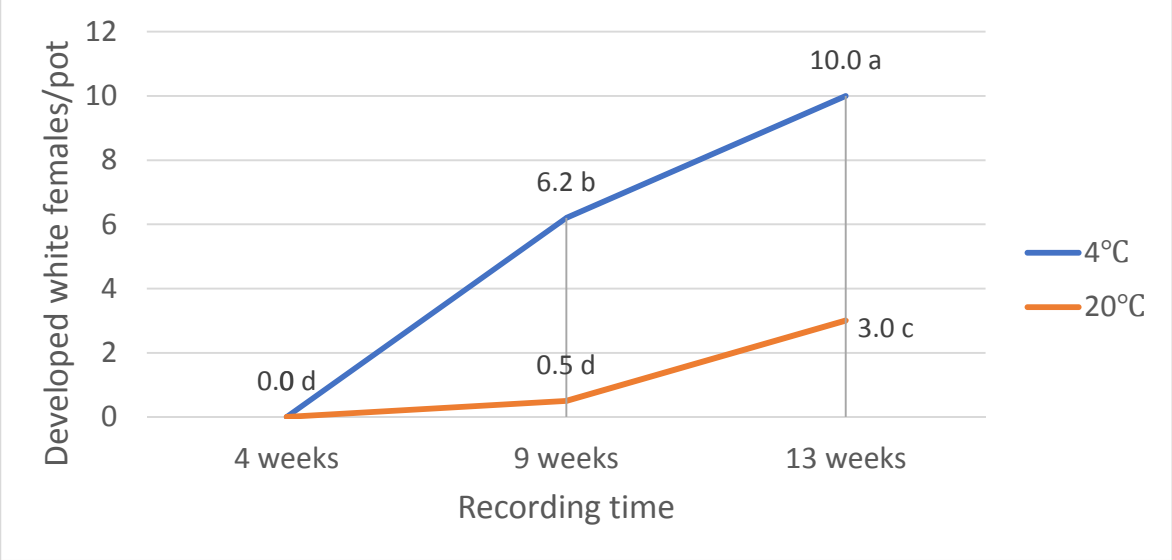
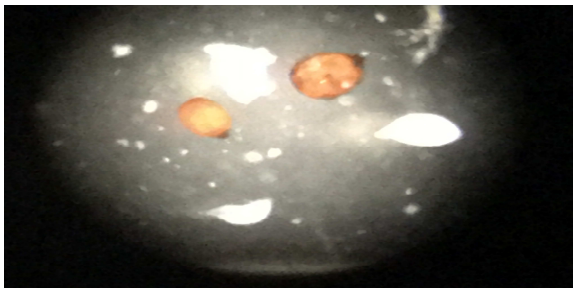
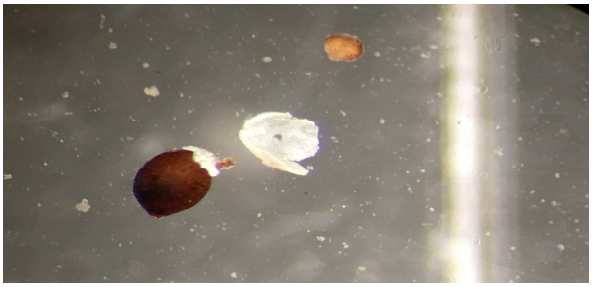


Figure 2.6. Interaction plot of the developed white females from dormant which were stored for eight weeks at 4°C or 20°C and then allowed to develop for4, 9, and 13 weeks after emergence of seedlings. Numbers followed by the same letter are not significantly different at α 0.05. The Y-axis represents the number of white females, and the X-axis represents the recording times (second experiment).

Appendix

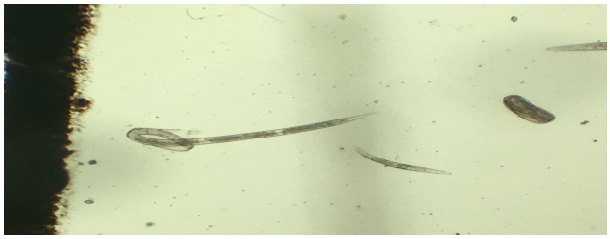


A



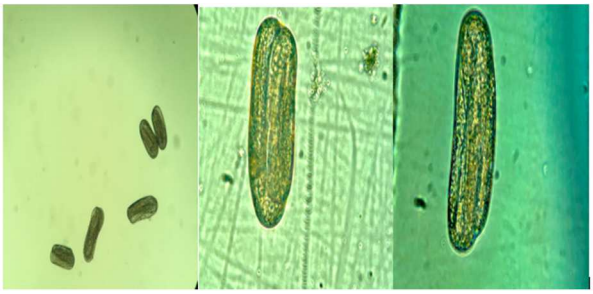
B

C



D

E



F

G

H

Appendix 1. Pictures with camera of 1.22 µm pixel size of cones shapes were taken under *Leica* light microscopes Different life stages of *Heterodera avenae*. A: White and brown females. B: shred white coat. C: failed hatched J2. D: newly hatched J2. E: half hatched J2. F: Unhatched J2.G: male. H: third stage J3. Magnification 10X * 20X = 200X / 0.35mm (350 microns).

The GLM for the two-weeks storing period				
White females	d.f	MS	F	P-Value
Model	8	15.04	62.24	<.0001
Error	15	0.24		
Corrected error	23			
Temperature	1	2.04	8.45	0.0108
Recording time	2	57.04	236.04	<.0001
Temperature*Recording time	2	2.04	8.45	0.0035
J2	d.f	MS	F	P-Value
Model	8	5887.50	9.50	0.0001
Error	15	620.00		
Corrected error	23			
Temperature	1	20416.66	32.93	<.0001
Recording time	2	10912.50	17.60	0.0001
Temperature*Recording time	2	279.16	0.45	0.6458
Hatching%	d.f	MS	F	P-Value
Model	8	9.77	13.19	<.0001
Error	15	0.74		
Corrected error	23			
Temperature	1	40.30	54.38	<.0001
Recording time	2	16.49	22.25	<.0001
Temperature*Recording time	2	0.02	0.03	0.9729
The GLM for the four-weeks storing period				
White females	d.f	MS	F	P-Value
Model	8	20.45	31.75	<.0001
Error	15	0.64		
Corrected error	23			
Temperature	1	54.00	83.79	<.0001
Recording time	2	40.66	63.10	<.0001
Temperature*Recording time	2	14.00	21.72	<.0001
J2	d.f	MS	F	P-Value
Model	8	36795.83	88.61	<.0001
Error	15	415.27		
Corrected error	23			
Temperature	1	266704.16	642.23	<.0001
Recording time	2	11329.16	27.28	<.0001
Temperature*Recording time	2	2179.16	5.25	0.0187

Hatching%	d.f	MS	F	P-Value
Model	8	62.22	104.75	<.0001
Error	15	0.59		
Corrected error	23			
Temperature	1	447.20	754.34	<.0001
Recording time	2	20.40	34.43	<.0001
Temperature*Recording time	2	5.94	12.27	0.0007
The GLM for the eight-weeks storing period				
White females	d.f	MS	F	P-Value
Model	8	20.45	31.75	<.0001
Error	15	0.64		
Corrected error	23			
Temperature	1	54.00	83.79	<.0001
Recording time	2	40.66	63.10	<.0001
Temperature*Recording time	2	14.00	21.72	<.0001
J2	d.f	MS	F	P-Value
Model	8	36795.83	88.61	<.0001
Error	15	415.27		
Corrected error	23			
Temperature	1	266704.16	642.23	<.0001
Recording time	2	11329.16	27.28	<.0001
Temperature*Recording time	2	2179.16	5.25	0.0187
Hatching%	d.f	MS	F	P-Value
Model	8	62.22	104.75	<.0001
Error	15	0.59		
Corrected error	23			
Temperature	1	447.20	754.34	<.0001
Recording time	2	20.40	34.43	<.0001
Temperature*Recording time	2	4.21	7.11	0.0067

Appendix 2. The GLM of SAS containing model fit, degree of freedom (df), means squares (MS), F value (F), and P- value of the white females, J2 and hatching percentage of the dormant eggs that were stored for 2 to 8 weeks at 4℃ and 20℃ and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (first experiment).

The GLM for the two-weeks storing period				
White females	d.f	MS	F	P-Value
Model	8	21.50	6.74	<.0001
Error	15	3.18		
Corrected error	23			
Temperature	1	13.50	4.23	0.051
Recording time	2	67.16	21.06	<.0001
Temperature*Recording time	2	6.50	2.04	0.161
J2	d.f	MS	F	P-Value
Model	8	2172.91	3.98	0.011
Error	15	545.27		
Corrected error	23			
Temperature	1	3037.50	5.57	0.033
Recording time	2	5887.50	10.80	0.0001
Temperature*Recording time	2	162.50	0.30	0.746
Hatching%	d.f	MS	F	P-Value
Model	8	3.68	4.40	0.006
Error	15	0.83		
Corrected error	23			
Temperature	1	5.13	6.14	<0.0001
Recording time	2	10.13	12.12	<.0001
Temperature*Recording time	2	0.32	0.39	0.685
The GLM for the four-weeks storing period				
White females	d.f	MS	F	P-Value
Model	8	51.25	38.44	<.0001
Error	15	1.33		
Corrected error	23			
Temperature	1	24.00	18.00	<.0001
Recording time	2	182.00	136.50	<.0001
Temperature*Recording time	2	10.50	7.87	<.0001
J2	d.f	MS	F	P-Value
Model	8	4750.00	7.08	<.0001
Error	15	1052.22		
Corrected error	23			
Temperature	1	2016.66	1.92	0.186
Recording time	2	25266.66	24.01	<.0001
Temperature*Recording time	2	2516.66	2.39	0.125

Hatching%	d.f	MS	F	P-Value
Model	8	13.86	9.52	<.0001
Error	15	1.45		
Corrected error	23			
Temperature	1	4.16	2.86	0.112
Recording time	2	46.32	31.83	<.0001
Temperature*Recording time	2	5.62	3.86	0.004
The GLM for the eight-weeks storing period				
White females	d.f	MS	F	P-Value
Model	8	42.33	18.52	<.0001
Error	15	2.28		
Corrected error	23			
Temperature	1	108.37	47.41	<.0001
Recording time	2	84.54	36.98	<.0001
Temperature*Recording time	2	27.87	12.19	<.0001
J2	d.f	MS	F	P-Value
Model	8	8385.14	2.83	0.070
Error	5	3525.27		
Corrected error	23			
Temperature	1	2204.16	0.63	0.440
Recording time	2	18612.16	5.28	0.018
Temperature*Recording time	2	11304.16	3.21	0.061
Hatching%	d.f	MS	F	P-Value
Model	8	34.28	12.93	<.0001
Error	15	2.65		
Corrected error	23			
Temperature	1	44.82	16.91	<.0001
Recording time	2	51.18	19.31	<.0001
Temperature*Recording time	2	59.85	22.85	<.0001

Appendix 3. The GLM of SAS containing model fit, degree of freedom (df), means squares (MS), F value (F), and P- value of the white females, J2 and hatching percentage of the dormant eggs that were stored for 2 to 8 weeks at 4℃ and 20℃ and then allowed to develop for 4, 9, and 13 weeks after emergence of wheat seedlings (second experiment).