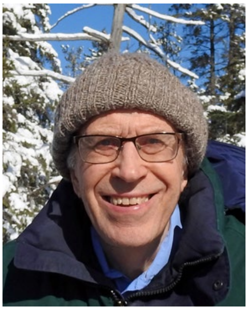
Wider aspects of a career in entomology.
16. Exploring insect cold hardiness

Hugh V. Danks



This series of articles outlines some ancillary aspects of my entomological career, for the potential amusement of readers. It reports the sometimes unexpected challenges of working in new places and in the real world, an approach that serves also to expose some conclusions about research and other entomological activities and some information about insects and their environments.

My early work in Canada focussed on the cold hardiness of chironomid midges from shallow ponds in temperate and arctic regions (see ESC *Bulletin* **50**: 50, 173). Those studies showed that many chironomid larvae tolerate freezing itself—unlike most cold-hardy insects, which supercool, preventing ice from forming in their bodies. Winter survival in chironomids depends too on further adaptations (including special winter cocoons), as well as on habitat selection and other ecological traits. Moreover, most temperate ponds were much less cold in winter than might have been anticipated.

I continued to study insect cold hardiness throughout my career, although my own cold hardiness seemed to diminish with time! A propensity to synthesize relevant information from the literature[1] was encouraged in particular because other commitments reduced the feasibility of fieldwork and experiments. Nevertheless, a few modest research projects provided stimulus and context for my reviews, even though the research itself was not always complete enough to submit for publication.

Large and flowing aquatic habitats were of particular interest because winter conditions differ so much from the shallow ponds already examined, yet chironomid midges are equally abundant. However, lakes (e.g., Figure 1) are often hard to access, troublesome to sample in cold weather, and unsafe when incompletely frozen. For example, even "solid" ice seldom freezes uniformly, snow cover slows down freezing, and ice that has thawed and refrozen is only half as strong as clear new ice. Sampling is hampered by intermittent melts, causing water (including seepage from the shore) to refreeze into the surface snow, and by ice shoves, which pile up ice at the edge of the largest lakes. Even in summer, deep central areas are challenging to sample effectively, and shoreline substrates are disturbed by waves.

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Figure 1. A frozen lake in Ontario.

Running water presents additional problems. Water movement hinders ice formation, and flowing water steadily erodes and weakens existing ice from below, making

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[1] Even some of my early papers devoted as much space to discussions as to results!

Hugh Danks (hughdanks@yahoo.ca) retired in 2007 after many years as head of the Biological Survey of Canada. In that role, he helped to coordinate work on the composition and characteristics of the arthropod fauna of the country, and to summarize the results. In addition, his research studied cold hardiness, diapause, and other adaptations to seasonality in northern regions.

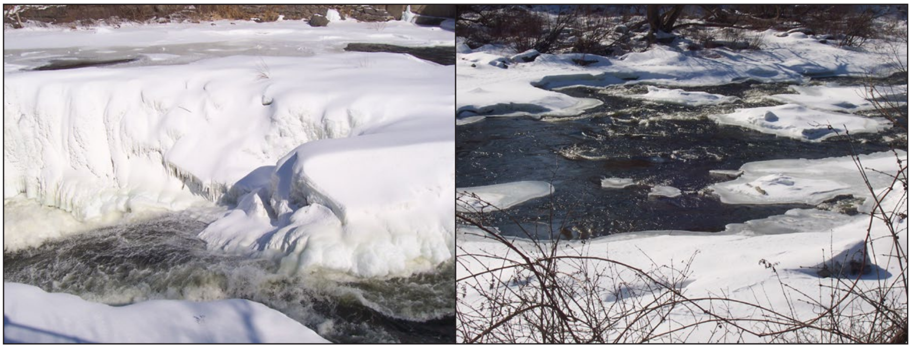

Figure 2. Two midwinter views of a fast river, largely unfrozen despite very low temperatures.

rivers treacherous (Figure 2). In spring, the thaw generates fragments of ice (Figure 3) and massive run-offs, which may destroy stream nets and other equipment.

These impediments can be overcome, but only with expensive logistic support (including power boats), and sampling equipment that can cope with deep or fast-flowing water. Clearly, it was both impracticable and dangerous to work alone—especially when armed only with a canoe paddle!

Figure 3. Ice fragments on a large river in spring.

Terrestrial habitats can be accessed more easily, although my initial overland travel in snowshoes was relatively slow (see ESC *Bulletin* **50**: 54). Skiing is supposedly more efficient, but my first exposure was as a beginner in a downhill skiing class, with skis that were far less advanced than they are today. Made of fibreglass with metal edges, those thick, heavy, and virtually inflexible planks were hard to control, despite massively engineered bindings and boots of bone-crushing rigidity. During my lessons, stories emerged about beginners who had broken their legs in slow twisting falls—whilst merely standing around in their skis listening to the instructor. I tried cross-country skiing instead, which proved more useful.

In the woods, most winter habitats are well protected beneath the excellent insulation provided by snow (cf. Figure 4). Safeguarded in this way, many species overwinter under leaves and logs, or in the soil. Some spiders and other arthropods even remain active at the insulated ground surface throughout the winter. Fallen trees (Figure 5) shelter some cold-hardy insects, including

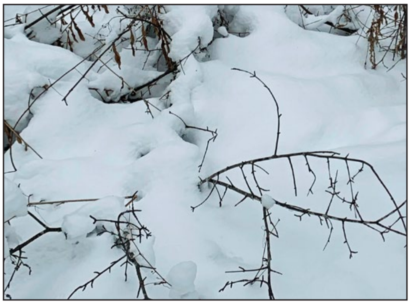

Figure 4. Snow above fallen leaves trapped around shrubs, providing protected overwintering habitats for many insects.

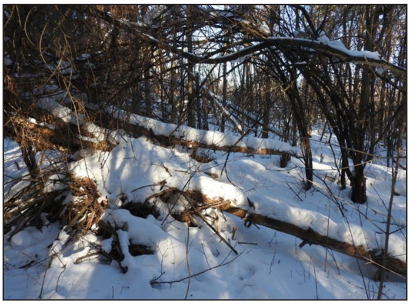

Figure 5. Fallen logs in winter.

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Figure 6. The ichneumonid *Ichneumon laetus,* which overwinters under bark. Length about 1.4 cm.

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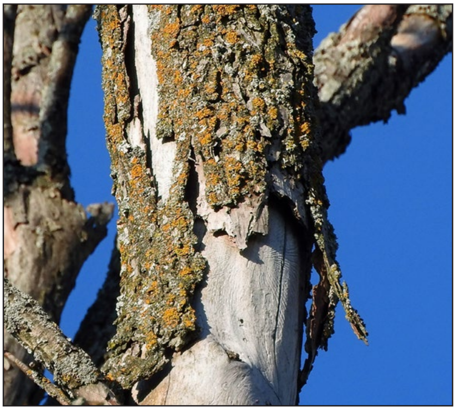
Figure 7. Larvae of the pyrhochroid beetle *Dendroides canadensis*, which overwinters under bark. Length up to about 2 cm.



H. Danks

Figure 8. Loose bark on a dead tree trunk above the snow.

Andy Reago & Chrissy McLarren (CC BY 2.0)

Figure 9. The depressariid [formerly oecophorid] moth *Agonopterix pulvipennella*, which overwinters under bark above the snow. Length about 1 cm.

ichneumonids (Figure 6) and beetle larvae (Figure 7).

Habitats under the loose bark of dead standing trees (Figure 8) become extremely cold and dry, and insects overwintering there should be particularly well adapted to harsh conditions. I examined the habitats, identified their characteristic properties, collected specimens—adults of a few species of ichneumonids and flat-bodied moths (e.g., Figure 9), and adults and larvae of several other taxa—and integrated findings from the literature. That information allowed me to design a complex sheet to record data on the temperature, moisture content, orientation, concealment, and other features of potential overwintering sites, as well as the location, posture, and behaviour of overwintering insects. Two or three species showed modest correlations, such as a preference for north-facing or for south-facing sites. However, despite long hours of searching, too few specimens were found to allow meaningful analyses. Some years later, uncomfortable memories of those labyrinthine data sheets were stirred by certain German authors, whose papers (on other subjects) presented meagre data but paid fanatical attention to minutely detailed classifications!

Outside the woods, the seed heads of cattails are abundant and fully exposed to winter cold (Figure 10). Heads that turn ragged and look "moth-eaten" may indeed house caterpillars of the shy cosmet moth (Figure 11). However, the heads are frayed not only by the caterpillars (which produce silk that hinders the dispersal of seeds), but also by normal seed dispersal, and damage by chickadees (which search for the larvae by pulling the heads apart).

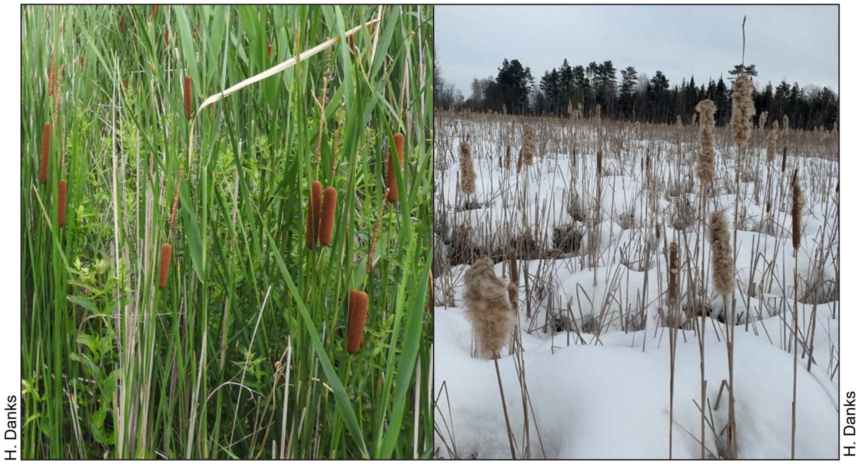


Figure 10. Cattail seed heads: in summer (L); and in winter (R). Seed-head length about 20 cm.

A given seed head may contain only a few individuals, and birds or parasitoids may already have found them. In any case, the larvae are difficult to sort out from the tufts of cottony hairs that serve to disperse the seeds. A typical head contains more than 200 000 seeds, so careless handling causes thousands of fine tufts to float off into the air. Consequently, obtaining and studying the tiny caterpillars was laborious, and detailed experiments on their cold hardiness did not prove worthwhile[2]. That project nevertheless confirmed the value of considering information about habitats, life cycles, parasites, predators, and so on, before focussing on some limited aspect of cold hardiness.

During the project, a fellow scientist was kind enough to ask their technician to help me sort out the larvae. Unfortunately, the technician specialized in work avoidance. He was assigned relatively few tasks, because his meagre efforts often saved a researcher less time than would be needed to instruct him[3]. Perhaps a project on cattails would have been feasible if he had been diligent, like most employees[4].

Stems of goldenrod are exposed above the snow in winter, and several insect species make galls on them. These abundant plants (Figure 12) in fact comprise many species of *Solidago*, about

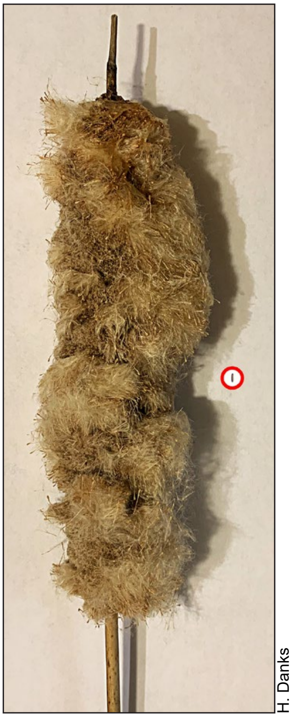


Figure 11. A cattail seed head collected in winter. Extracted from that same seed head, and circled mid-R at the same scale, is an overwintering larva of the shy cosmet moth (cattail seedhead moth), the cosmopterygid *Lymnaecia phragmitella*, about 4 mm long.

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[2] Apparently, no detailed study of the cold hardiness of this species has yet been made. It is widely distributed across the world, including areas with warmer winters. In the British Isles it is known as the bulrush cosmet moth.

[3] Regrettably, his supervisor had avoided the unpleasant tasks of proper documentation and critical annual appraisals, even though his behaviour was well known. After several years of "satisfactory" appraisals, it was difficult to hold him to account.

[4] However, one other slacker spent hours busily walking along the corridors with documents, as though he was using the library or transferring data—but the sheets of paper he carried were merely props to help him avoid real work!

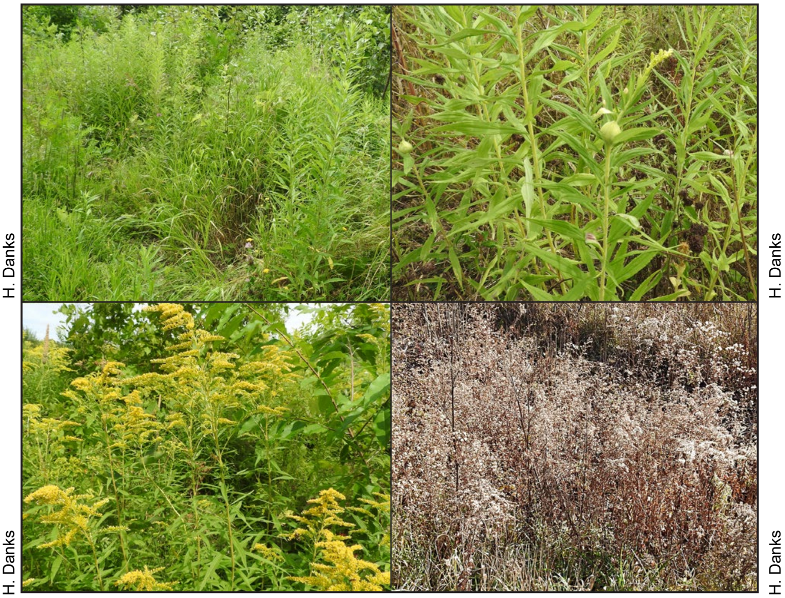

Figure 12. Seasonal development in stands of goldenrod. L and R from top to bottom: growing plants; plants with galls; plants in flower; dead plants and seed heads in early winter.

a dozen of which are common in Ontario. They are characteristic of disturbed ground and among the first species to invade after disturbance. The perennial plants multiply through rhizomes as well as seeds (and can regenerate after fire), so they persist for a number of years until succeeded by shrubs. However, succession became more difficult to track near Ottawa as productive fields were replaced by housing subdivisions, shopping malls, and country homes!

The most common galls belong to the goldenrod gall fly, which spends much of its life inside the gall (Figure 13) and overwinters as a fully grown larva (Figure 14)[5]. The larva makes an exit tunnel nearly to the surface of the gall before overwintering.

Much work on cold hardiness has been done on the larvae, which survive when frozen solid. Large quantities of the cryoprotectant glycerol (and some sorbitol) are sequestered for the winter. Larvae survive less well and produce less fecund adults when (despite greatly depressed respiration rates) their energy reserves are depleted by warm

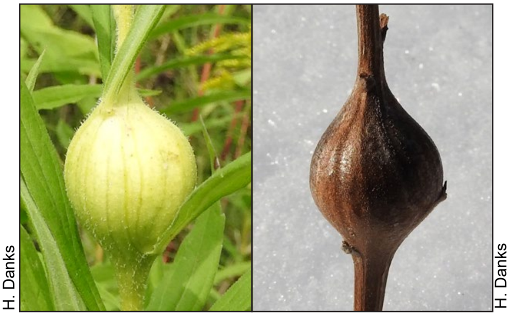

Figure 13. Galls of the goldenrod gall fly, the tephritid *Eurosta solidaginis*: in summer (L); and in winter. Gall diameter about 2 cm.

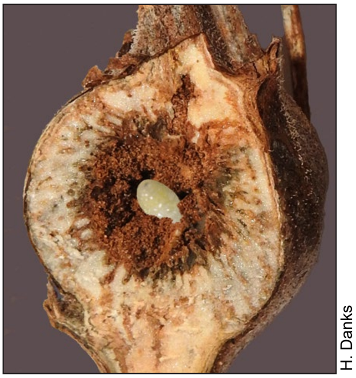

Figure 14. Winter gall of the goldenrod gall fly, opened to show the larva within.

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[5] Adults emerge in late spring and oviposit in the growing tips of goldenrod.

interludes, which may also subject them to freeze-thaw cycles.

The gall flies are part of a complex system, just like the cattail moth. For example, they are common on only three of the species of *Solidago*, and their preference for those species varies regionally. The gall fly appears to invade most goldenrod stands relatively rapidly, but its local abundance is by no means random. Some long-established sites build up large populations (e.g., Figure 15).

Figure 15. Winter stems of goldenrod, showing the potential local abundance of galls.

A number of parasitoids and a mordellid beetle predator attack the larvae in summer. Chickadees and downy woodpeckers peck into the galls during winter, and are reported to tap on each gall to locate the exit channel and so retrieve the morsel inside more easily. The attack of parasitoids is more successful on smaller galls, but birds prefer larger ones. Some parasitoids overwinter as larvae in the galls (Figure 16), and must have comparable cold hardiness.

Figure 16. Larva of the eurytomid *Eurytoma gigantea* (Chalcidoidea) inside a gall of the goldenrod gall fly.

The elliptical galls of the goldenrod gall moth also occur on goldenrod (Figure 17). The larva overwinters in a silk-lined cavity just below the gall[6]. In this species, substantial amounts of glycerol act as an antifreeze down to temperatures close to –40°C. The moth too belongs to a community of parasitoids and other associates, although apparently birds do not seek the larvae.

Most ecological factors have not been integrated with the physiological work. Presumably, cryoprotectants and energy reserves are affected by the size of the larva, in turn possibly correlated with the gall size that influences natural enemies. However, understanding the relationships among these and other factors would require extensive research, which was not feasible for me.

Nevertheless, in due course my interest in the gall fly highlighted another aspect of its winter survival, and showed that joint projects, even limited ones, can have great value. One possible feature of frozen insects came to light during my early studies in the high arctic, when chironomid larvae collected from frozen ponds were embedded for cytological examination by

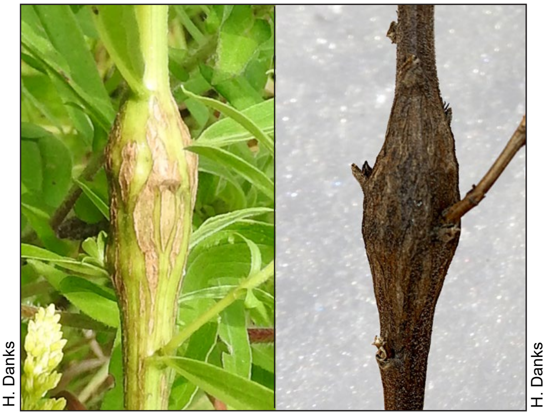

Figure 17. Galls of the goldenrod gall moth, the tortricid *Epiblema scudderiana*: in summer (L); and in winter. Maximum gall diameter about 1 cm.

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[6] The gelechiid moth *Gnorimoschema gallaesolidaginis* also produces an elliptical gall on goldenrod, but the species overwinters as an egg, and the galls are empty in winter.

my colleague Bob Byers (see ESC *Bulletin* **50**: 175). Back in Ottawa, he saw that the mitochondria appeared to have broken down. Bob had no way to follow up or verify the observation[7], and thought that it might be an artifact in material prepared under difficult field conditions. Eventually, however, mitochondrial degradation was documented independently by Olga Kukal in the arctic woollybear (Figure 18); and later still, Olga and I collaborated in a project on that species.


O. Kukal

Figure 18. Arctic woollybear, the larva of thelymantriine erebid *Gynaephora groenlandica*. Caterpillars overwinter many times during the long life-cycle. Length about 3.5 cm.

Another cooperative project, with David Levin in his laboratory at the University of Victoria, British Columbia, used modern techniques to examine mitochondria in both the goldenrod gall fly and the arctic woollybear. Seasonal degradation of mitochondria was confirmed in the caterpillars, with concomitant reduction of mitochondrial DNA. Similar changes took place in gall-fly larvae, and mitochondrial DNA and respiration rates increased very rapidly when larvae were warmed. These findings suggest that mitochondrial degradation may be widespread, at least in species that tolerate freezing like the gall fly, the arctic woollybear, and arctic chironomids[8]. Perhaps, by suspending metabolism, it relates to energy use or molecular protection, not simply to reduced respiration.


Donald Hobern (CC BY 2.0)

Figure 19. Pupa of the winter moth, the geometrid *Operophtera brumata*. Length about 1 cm.

My earlier cooperative venture at the same university involved a visit to the laboratory of Richard Ring, to follow up his initial (unpublished) finding of glycerol in pupae of winter moth (Figure 19) and Bruce spanworm (*Operophtera bruceata*), which remain dormant for several months during the dry summer. I planned to generate additional data using high-pressure liquid chromatography (HPLC)[9], but was not able to calibrate the chromatograph. A graduate student (who had used the same equipment successfully) tried to assist me, but made no progress either. Only then did we discover that the machine had recently been made available to a large class of untrained undergraduate students, and was now unusable!

Despite this setback, the existing data for pupae prompted me to synthesize published information to highlight the fact that key adaptations for resistance to dehydration in summer are the same as those known for cold hardiness in winter: the production of various solutes (“cryoprotectants”) such as glycerol, adjustments of water content, and selection of sheltered habitats, suggesting that many of the physiological and ecological adaptations interpreted as conferring cold hardiness serve too to ensure survival in dry environments.

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[7] Moreover, soon afterwards the Canada Department of Agriculture discontinued its support of projects on insect cold hardiness.

[8] Later work by others showed no such response in the goldenrod gall moth, which avoids freezing by supercooling.

[9] Now referred to as high-performance liquid chromatography.

A subsequent joint project documented the presence of trehalose in pupae of the winter moth, and emphasized its potential roles. This sugar has a number of significant properties, in addition to its ability (like many cryoprotectants) to depress the freezing point of solutions. For example, trehalose has low reactivity, and when dried to very low water levels forms a glass, even at biologically relevant temperatures. Glasses are amorphous rather than crystalline, a structure that appears to prevent proteins and other substances in the glass from undergoing distortions or harmful chemical changes. Trehalose can therefore protect tissues against injury from both desiccation and freezing, as reported in diverse organisms.

The fact that key adaptations resist both dryness and cold is now widely accepted, prompting further research, and confirming that even small projects, especially cooperative ones, can produce useful results.

My short stay in Japan in 2004–2005 included a minor role in a project on the striped rice stem borer (the crambid *Chilo suppressalis*). The species overwinters as a fully grown larva, which accumulates glycerol (and some trehalose) and can survive when frozen. Work with fat-body cells showed that water-transport proteins (aquaporins, located in the cell membrane) are essential to survival, by mediating the replacement of water by glycerol in the tissues. This function of aquaporins is now well established, and even includes research on a familiar species: the goldenrod gall fly[10].

My explorations of cold hardiness showed the value of cooperative work; and they generated additional conclusions. First, of course, there are practical barriers to field studies in winter—apart from preventing frostbite, avoiding temporary or permanent immersion in partly frozen water bodies, and offsetting the ways in which Murphy's Laws of Fieldwork conspire to hamper research. Taking samples and recording data are particularly challenging in heavy or blowing snow—and any tool briefly laid down soon disappears. Labelling has to be foolproof even in thick gloves, or when samples just taken under the ice of ponds and lakes are wet. In the days before compact audio devices, it was difficult to record notes, especially detailed written descriptions rather than simple measurements.

Documenting information with photographs is troublesome in winter. Gloves hinder adjustment and activation of the camera, and equipment or batteries may function poorly in cold weather. In snowy places, especially in sunshine, photographs can easily be overexposed, producing highly

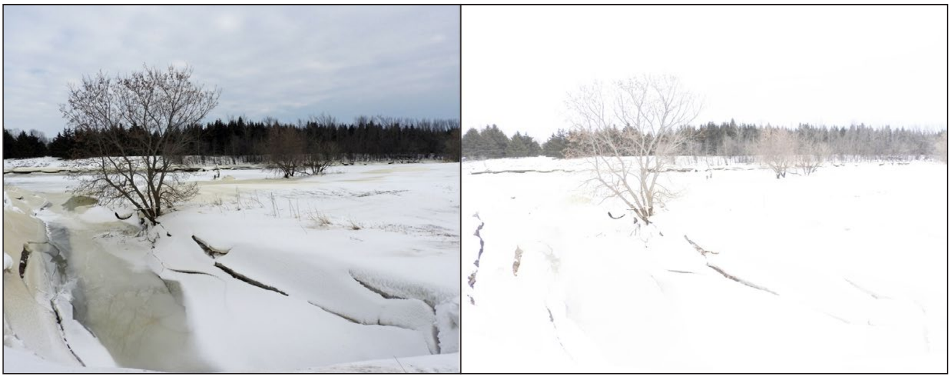
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Figure 20. A snow-covered creek in winter: photographed with adequate exposure (L); and overexposed (R).

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[10] Consistent with an obsession with goldenrod, the caterpillar of *Agonopterix pulvipenella*, the moth shown in Figure 9, feeds on that plant.

saturated images with little definition (cf. Figure 20)[11]. People seeing the most extreme examples might wonder why anyone had bothered to photograph a white bedsheet …

Another conclusion from my explorations is that staying in the same field of study has many advantages. Becoming familiar with the full extent of a subject area reveals its complexity, and is useful to explain specific findings as well as suggest fruitful lines of enquiry.

A researcher need not investigate every aspect personally, of course, but a wide approach helps to identify projects that are feasible, productive, and informative. Moreover, realizing these benefits by integrating information already published is possible without additional funding!

Considering insect cold hardiness as broadly as possible reveals that the habitat conditions, life cycles, and physiology of species are interlocked. Therefore, understanding the full significance of any one adaptation requires detailed knowledge about others, a conclusion that must apply to scientific studies in general.

For example, overwintering sites are not in a static deep freeze during the winter. All of them—even under snow—warm up and cool down as the weather changes (e.g., ESC *Bulletin* **50**: 50). Therefore, more extensive records than might be expected are needed to discover the winter conditions that insects actually have to withstand. In addition, the spring melt of snow and ice is a powerful and dynamic process that interacts with insect habitats.

Each species overwinters in particular stages and places, but these details depend not only on exposure to cold (and other conditions), but also on protection from natural enemies, and on patterns of development and reproduction in relation to the seasons. Therefore, work that is too narrowly focussed is difficult to interpret.

Adaptations of physiology and biochemistry show many common themes, such as widely distributed haemolymph solutes. However, relating the great differences among species to specific features of their natural environments has only just begun.

These wide perspectives prompted and supported my reviews of cold hardiness, life-cycle control, and allied themes, and towards the end of my career led me to re-examine the winter adaptations of aquatic insects, the subject of my early research. Impediments to the study of large and flowing aquatic habitats were finally overcome … by synthesizing the literature without facing the difficulties of winter fieldwork!

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[11] Such errors are less frequent with the sophisticated autoexposure capabilities of modern cameras.