Chronotoxicology of Agricultural Insect Pests: Does the Time of Pesticide Application Matter?

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ABSTRACT

Insect pest management has traditionally focused on choosing an effective insecticide, determining an appropriate dose and selecting the correct method of application. A less explored factor, however, is time. Chronotoxicology is the study of how the biological time of exposure influences the response of an organism to a toxic substance. In insects, many physiological and behavioural processes operate according to approximately 24 hours circadian rhythms. Feeding, locomotion, hormone production, detoxification, gene expression and metabolism may therefore change between day and night. These changes can alter the susceptibility of agricultural pests to insecticides even when the same insecticide is applied at the same concentration. Understanding such temporal variation could improve insecticide effectiveness, reduce unnecessary pesticide use and support more precise and environmentally compatible pest management.

INTRODUCTION:

Agricultural pest management generally asks three questions: which insecticide should be used, at what concentration, and how should it be applied? Chronotoxicology introduces another important question: when should it be applied? The term chronotoxicology combines '*chrono*' meaning 'time' with toxicology, the study of harmful effects caused by chemicals. In entomology, it describes changes in the

toxicity or effectiveness of a chemical according to the time at which an insect is exposed. The concept is closely linked with circadian rhythms, biological cycles that repeat approximately every 24 hours and are synchronized primarily by environmental cues such as light and darkness (Fig 1). Circadian clocks regulate many insect functions, including feeding, movement, hormone secretion, metabolism and detoxification (Hooven *et al.,* 2009).

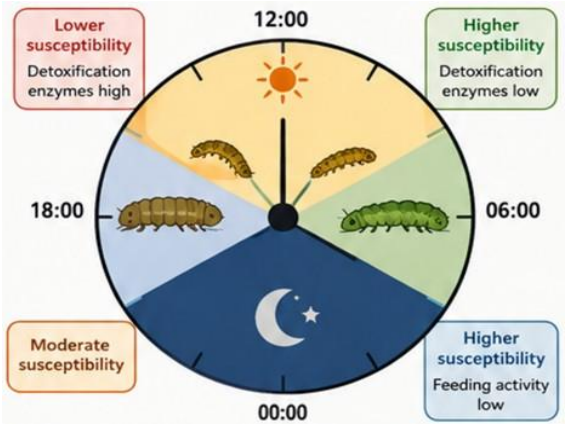


Fig 1: Circadian variation in insecticide susceptibility

This means that an insect exposed to an insecticide in the morning may not respond exactly like another insect exposed to the same dose at night. The chemical has not changed, but the physiological condition of the insect has. Consequently, pesticide efficacy should not always be considered constant throughout the day.

What is circadian rhythm in insects?

A circadian rhythm is an internally controlled biological cycle of approximately 24 hours. Although environmental factors such as light and temperature help synchronize the clock, many rhythms continue even when external conditions are kept constant. Researchers therefore distinguish between a diel rhythm, which follows the external day-night cycle, and a circadian rhythm, which persists under constant conditions because it is driven by an internal biological clock (Hardin, 2005).

In laboratory studies, researchers often use the term Zeitgeber Time (ZT). ZT0 usually represents the time when lights are switched on and ZT12 represents the beginning of the dark period in a 12-hour light / 12-hour dark cycle. Experiments performed under continuous darkness may use Circadian Time (CT) to determine whether a response continues without external light cues. The importance of this distinction is substantial. If insecticide susceptibility continues to fluctuate under constant darkness, the response is more likely to be controlled by the insect's endogenous circadian system rather than simply being a reaction to daylight.

Why does insecticide susceptibility change with time?

Several biological mechanisms may contribute to chronotoxicity.

1. **Detoxification enzymes:** One of the most important mechanisms involves enzymes that metabolize foreign chemicals. Insects use detoxification systems containing cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), carboxylesterases (CarEs) and related enzymes to modify or eliminate toxic compounds. The amount or activity of these enzymes can vary over the 24-hour cycle. In *Musca domestica*, for example, susceptibility to beta-cypermethrin changed according to time of day and was accompanied by daily rhythms in P450 and carboxylesterase activity as well as rhythmic expression of selected detoxification genes (You *et al.,* 2022). Such findings suggest that an insect may be better equipped to detoxify a pesticide during one part of the day than another.

2. **Feeding and behavioural rhythms:** Insect behaviour also strongly influences pesticide exposure. Many agricultural pests are not equally active throughout the day.

Nocturnal insects may feed intensively after sunset and remain concealed during daylight. If an insecticide acts primarily through ingestion or contact, the timing of exposure relative to feeding and movement can influence the amount of toxicant encountered. A striking example is *Spodoptera litura*, whose larvae feed mainly at night but show higher expression of several digestive and detoxification related genes during the day. Experimental exposure to imidacloprid produced greater effects during the night than during the daytime, demonstrating that behavioural and metabolic rhythms can intersect to produce chronotoxicity (Zhang *et al.,* 2021).

3. **Gene expression:** The molecular clock controls rhythmic expression of many genes. The core circadian system includes clock associated genes such as *period*, *timeless*, *clock* and *cycle*. Some genes associated with xenobiotic metabolism may also show temporal expression patterns. In *Aedes aegypti*, temporal variation in permethrin response was associated with rhythmic expression of the clock gene *period* and the detoxification associated gene *CYP9M9*. Silencing period altered both gene expression and insecticide resistance, providing evidence for a connection between the circadian clock and insecticide susceptibility (Yang *et al.,* 2010).

Evidence From Agricultural and Other Pest Insects

Chronotoxicity is not restricted to one insect order or one chemical class. Research has demonstrated daily variation in insecticide responses in several species.

Early experimental work in *Drosophila melanogaster* showed that susceptibility to some pesticides varied during the day. Propoxur and fipronil produced stronger daily differences than deltamethrin and malathion, demonstrating an important point: chronotoxicity is not necessarily the same for every pesticide (Hooven *et al.,* 2009).

In the German cockroach, *Blattella germanica*, susceptibility to permethrin exhibited a pronounced daily rhythm. Gene expression of the GST gene *BgGSTD1* also oscillated, and RNA interference targeting this gene altered nighttime susceptibility, supporting a functional relationship between detoxification and temporal insecticide response (Lin *et al.,* 2014).

Similarly, the common bed bug *Cimex lectularius* showed a circadian pattern in deltamethrin susceptibility, with peak tolerance occurring during a particular phase of the light – dark cycle. The same approximate phase persisted under constant darkness, indicating involvement of an endogenous circadian clock. Interestingly, this pattern was not observed for imidacloprid, again emphasizing that chronotoxicity depends on the insecticide as well as the insect (Khalid *et al.,* 2019).

Research on *Drosophila suzukii* also demonstrated that chronotoxicity can be related to both activity patterns and detoxification gene expression. The species exhibited distinct seasonal daily activity patterns, while several detoxification genes showed rhythmic expression. Malathion susceptibility was greatest at a particular circadian phase, illustrating the potential value of matching chemical exposure with pest biology (Hamby *et al.*, 2013).

Chronotoxicology and Agricultural Pest Management

The practical implication is straightforward: the same pesticide does not necessarily produce the same biological outcome at every hour of the day.

Consider a hypothetical nocturnal caterpillar whose susceptibility is highest during the early night. Spraying immediately before its period of maximum susceptibility could increase the probability of successful control. Conversely, spraying when detoxification capacity is highest could reduce efficacy. However, timing should not be selected simply on the basis of whether the pest is active. The ideal application window should consider several factors simultaneously:

Pest activity + physiological susceptibility + insecticide persistence + environmental conditions + natural enemy activity.

This is especially important in Integrated Pest Management (IPM). A spray timed for maximum pest vulnerability could potentially reduce the amount of pesticide required. Better timing may also reduce repeated applications caused by poor efficacy, thereby lowering cost, residues and selection pressure for resistance. However, chronotoxicology should not be interpreted as a universal rule that "night spraying is always better" or "morning spraying is always better".

How can chronotoxicology be studied?

A typical experiment begins by maintaining insects under a controlled light – dark cycle, such as 12 hours light and 12 hours dark. Individuals are then exposed to the same insecticide concentration at several different times.

For example:

ZT0 → ZT4 → ZT8 → ZT12 → ZT16 → ZT20

Mortality, knockdown, feeding inhibition or other responses can then be measured.

For a deeper mechanistic study, researchers can measure:

- LC_{50} or LD_{50} at different times
- detoxification enzymes such as P450, GST and CarE
- expression of detoxification genes
- expression of clock genes
- feeding and locomotor activity
- larval growth and development
- pupation and adult emergence
- fecundity and fertility
- sublethal behavioural effects

Experiments under constant darkness are particularly useful because they help determine whether a rhythm is genuinely circadian rather than simply caused by the external light – dark cycle (Hooven *et al*., 2009; Khalid *et al.,* 2019).

Future Prospects

Chronotoxicology could eventually become part of precision pest management. Instead of applying pesticides only according to conventional schedules, farmers could potentially use information about pest activity, physiological susceptibility, weather and residual toxicity to identify better application windows. Future research should examine chronotoxicology in economically important crop pests under realistic field conditions. Another important area is the relationship between pesticide timing and beneficial insects. If pest susceptibility peaks when parasitoids and predators are relatively inactive, appropriately timed application could potentially improve pest suppression while reducing exposure to beneficial arthropods. The field also offers opportunities to investigate whether prolonged pesticide exposure alters circadian rhythms themselves and whether altered circadian regulation contributes to long term adaptation or resistance. Such questions connect

chronobiology with toxicology, insect physiology, molecular entomology and IPM.

CONCLUSION

Chronotoxicology provides a new way of thinking about insect pest management. Instead of treating insecticide action as a constant process, it recognizes that an insect's biological state changes throughout the day. Circadian regulation of detoxification enzymes, gene expression, feeding, movement, metabolism and endocrine physiology can influence how strongly a pest responds to the same chemical exposure. Evidence from several insect species demonstrates that pesticide susceptibility can vary significantly with biological time, although the pattern differs among species and insecticides. The central lesson is therefore not simply to spray during the day or night, but to understand the biological clock of the target pest and match management practices to it. Chronotoxicology could ultimately shift pesticide application from a simple chemical decision to a time optimized biological strategy. Such an approach has the potential to improve control efficiency, reduce unnecessary pesticide use and contribute to a more precise and sustainable future for agricultural entomology.

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