TRIPLE-IJ and SHIFT as a coupled framework for characterising entomopathogenic nematode performance under abiotic stress

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Abstract

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are widely recognised as promising biological control agents, yet the translation of laboratory-demonstrated virulence into consistent field-scale efficacy remains an unresolved challenge. A central reason for this gap is the fragmented way EPN performance is conventionally characterised: infectivity and reproductive output are measured as isolated endpoints, and the ecological fitness of infective juveniles (IJs) following cadaver emergence is rarely integrated into the same analytical framework. This paper proposes two conceptually linked frameworks to address this limitation. TRIPLE-IJ (Temperature Response in Infectivity and Progeny Leading to EPN-IJ dynamics) provides a structured, eight-parameter approach to characterising the dual effects of temperature on EPN performance, separating the infectivity phase (time to death, larval mortality, infection intensity, and proportion of larvae producing IJs) from the reproductive phase (latency to first emergence, emergence duration, IJ yield, and cadaver productivity). SHIFT (Stress Hurdles and IJ Foraging Traits) organises post-emergence ecological fitness across three major abiotic stress categories, namely desiccation, hypoxia, and oxidative stress, in relation to the foraging strategies governing host-seeking capacity. Integrated as a sequential two-gate

performance filter, the frameworks generate four biologically meaningful isolate archetypes that can guide screening, selection, and deployment decisions in biocontrol programmes. Both frameworks were derived retrospectively from empirical work on indigenous Nigerian EPN isolates evaluated against *Spodoptera frugiperda* but are proposed as broadly applicable tools for EPN characterisation across host systems and agroecological contexts. Their adoption as a minimum reporting standard would improve cross-study comparability and strengthen the evidence base for EPN deployment in tropical and subtropical biocontrol programmes, with particular relevance for sub-Saharan Africa where indigenous isolate screening is expanding rapidly.

Keywords: entomopathogenic nematodes; biological control; temperature stress; desiccation tolerance; foraging strategy; isolate characterisation; TRIPLE-IJ; SHIFT; sub-Saharan Africa

1. Introduction

The entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae occupy a unique position in biological pest control. As obligate insect parasites, they combine lethal pathogenicity with a free-living dispersal stage, the infective juvenile (IJ), which actively seeks hosts in the soil environment and kills them rapidly through the combined action of the nematode and its mutualistic bacterial symbiont (*Xenorhabdus* spp. in steinernematids; *Photorhabdus* spp. in heterorhabditids) (Kaya & Gaugler, 1993; Poinar, 1990; Dillman et al., 2012). Their broad host range, compatibility with integrated pest management strategies, safety to vertebrates and most non-target organisms, and suitability for mass production have sustained decades of research interest and commercial development (Grewal et al., 2005; Lacey et al., 2015; Georgis et al., 2006). Yet a persistent and well-documented gap remains: laboratory-demonstrated virulence does not reliably predict field-scale biocontrol outcomes (Gaugler & Kaya, 1990).

This gap is not incidental. It reflects a fundamental mismatch between the conditions under which EPN performance is conventionally measured and the abiotic reality that infective juveniles encounter following field application. Temperature is among the most consequential of these

abiotic factors, and its influence on EPN biology is neither simple nor unidirectional. Rather, temperature operates on two biologically distinct phases of the infection cycle simultaneously: the infectivity phase, during which IJs must locate, penetrate, and establish within a living host; and the reproductive phase, during which established nematodes develop, reproduce, and release the next generation of IJs from the host cadaver. These two phases respond to thermal gradients in ways that are not always congruent, and a thermal condition permissive for infection may be suboptimal for reproduction, or vice versa (Grewal et al., 1994; Hazir et al., 2001; Brown & Gaugler, 1996). Understanding EPN performance under temperature stress therefore demands that both phases be characterised together, using parameters that are independently measured and clearly defined.

Beyond temperature, IJs emerging from host cadavers face an additional suite of ecological stressors that determine whether the reproduced population can persist, disperse, and initiate new infection cycles. Desiccation is widely regarded as one of the principal constraints on EPN survival in field soils, particularly in tropical and semi-arid environments where soil moisture fluctuates markedly (Glazer, 2002; Patel et al., 1997; Shapiro-Ilan et al., 2014). Oxygen limitation in waterlogged or compacted soils presents a further challenge, as does oxidative stress arising from reactive oxygen species encountered during host immune responses and free-living soil persistence (Grewal et al., 2002; Maushe et al., 2023). Overlaid on these stress hurdles is the foraging strategy of the IJ, which determines its movement ecology and host encounter rate. The distinction between ambush foragers, which remain near the soil surface awaiting mobile hosts, and cruise foragers, which actively traverse the soil matrix in search of sedentary hosts, has direct consequences for how different isolates respond to and recover from ecological stress (Lewis et al., 1995; Lewis et al., 2006; Campbell & Gaugler, 1993).

Current approaches to EPN characterisation, however, seldom integrate these dimensions into a coherent analytical framework. Virulence bioassays typically report mortality and IJ yield as separate and often unconnected endpoints (Kaya & Gaugler, 1993; Lacey & Georgis, 2012). Stress tolerance assays are routinely conducted as standalone experiments, rarely linked back to the infectivity or reproductive data that would contextualise their ecological significance. The result is a body of literature rich in isolated measurements but limited in its capacity to generate holistic, comparable profiles of isolate performance (Georgis et al., 2006; Stuart et al., 2006). This limitation is particularly consequential in tropical regions of sub-Saharan Africa, where surveys of

indigenous EPN diversity are still in early stages and where the selection of isolates for biocontrol programmes depends on precisely the kind of integrative performance data that current frameworks do not provide (Abate et al., 2023).

The invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), provides a compelling context within which these limitations are directly consequential. First detected in Africa in 2016, *S. frugiperda* spread rapidly across sub-Saharan Africa, posing severe threats to maize production and rural food security (Goergen et al., 2016; Day et al., 2017; Rwomushana et al., 2018). The subsequent reliance on synthetic chemical insecticides has raised well-documented concerns regarding resistance development and environmental damage (Harrison et al., 2019; Guo et al., 2020). Studies have demonstrated meaningful virulence of both *Steinernema* spp. and *Heterorhabditis* spp. against larval instars of *S. frugiperda* under controlled conditions (Ratnakala et al., 2023; Patil et al., 2022), supporting their potential as biocontrol agents in maize agroecosystems. However, translating this potential into reliable field-scale efficacy requires precisely the integrative performance characterisation that the present frameworks provide.

This paper proposes two conceptually linked frameworks developed from empirical work with indigenous Nigerian EPN isolates against *S. frugiperda* (Okolo et al., 2025). The first, designated TRIPLE-IJ (Temperature Response in Infectivity and Progeny Leading to EPN-IJ dynamics), provides a structured, eight-parameter approach to characterising the dual effects of temperature on EPN infectivity and reproductive performance. The second, SHIFT (Stress Hurdles and IJ Foraging Traits), organises the ecological fitness of IJs across three major abiotic stress categories, desiccation, hypoxia, and oxidative stress, in relation to the foraging strategies that determine their host-seeking efficacy. Together, these frameworks offer an integrated platform for evaluating EPN isolate performance across the full arc from initial host infection through to post-emergence ecological persistence. It is argued that the adoption of such a coupled framework would improve the rigour and comparability of EPN characterisation studies and ultimately strengthen the evidence base for EPN deployment in tropical biocontrol programmes, particularly in sub-Saharan Africa where indigenous isolate screening is still in its early stages (Tarasco et al., 2023).

2. The TRIPLE-IJ Framework

2.1 Conceptual Rationale

The biological process by which an entomopathogenic nematode converts a living host into a reproductive resource is not a single event but a sequential two-phase process, and this distinction matters enormously when evaluating performance under abiotic stress. The first phase, infectivity, encompasses all events from initial host contact through IJ penetration, symbiont release, host death, and the establishment of nematodes within the haemocoel. The second phase, reproduction, encompasses the development of those established nematodes through successive generations, the production of the next IJ cohort, and the eventual emergence of progeny from the host cadaver. These two phases are biologically distinct, temporally separated, and physiologically regulated by different mechanisms; yet they share a common driver in the thermal environment (Grewal et al., 1994; Hazir et al., 2001).

The relationship between temperature and each phase does not follow a uniform gradient. Thermal optima for infection and establishment may differ from optima for reproduction, such that an isolate performing well at a given temperature in terms of host mortality may produce substantially fewer progeny at that same temperature than at a cooler or warmer point along the thermal gradient (Grewal et al., 1994; Brown & Gaugler, 1996). Critically, a reproductive failure following successful infection represents a complete loss of population recycling potential, regardless of how rapidly or completely the host was killed. Evaluating only one phase therefore yields an incomplete and potentially misleading characterisation of isolate performance under temperature stress. Both phases must be measured together, using parameters that are independently derived and clearly operationalised (Kaya & Gaugler, 1993; Gaugler, 2002).

TRIPLE-IJ formalises this dual-phase approach into an eight-parameter framework (Fig. 1). The acronym encodes the two phases directly: TRIP (T, R, I, P) captures the infectivity phase, and LEIJ (L, E, I, J) captures the reproductive phase. The parameters within each group were selected on the basis that each measures a distinct and independently quantifiable biological outcome, and that together they provide a complete characterisation of their respective phase under varying thermal conditions.

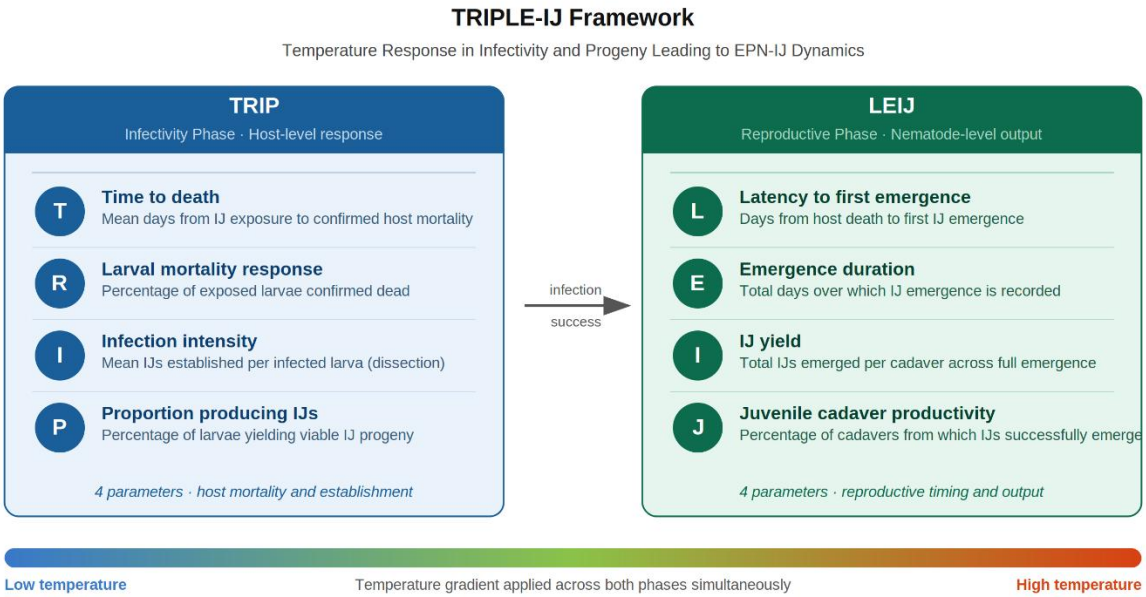

Fig. 1. **The TRIPLE-IJ framework**. Left panel (blue): four infectivity parameters of the TRIP phase, representing host-level responses to IJ infection. Right panel (teal): four reproductive parameters of the LEIJ phase, representing nematode-level reproductive output. The temperature gradient bar at the base indicates that both phases are profiled simultaneously across the same thermal range to produce a coupled isolate performance curve.

2.2 Parameter Definitions

The eight parameters of the TRIPLE-IJ framework are defined formally in Table 1, organised by biological phase.

Table 1. The eight parameters of the TRIPLE-IJ framework, organised by biological phase.

Phase	Letter	Parameter	Definition
Infectivity (TRIP)	**T**	Time to death	Mean number of days from IJ exposure to confirmed host mortality
	R	Larval mortality response	Percentage of exposed larvae confirmed dead within the observation period
	I	Infection intensity	Mean number of IJs established per infected larva, determined by dissection
	P	Proportion producing IJs	Percentage of exposed larvae that yield viable IJ progeny upon cadaver processing

Reproduction (LEIJ)	**L**	Latency to first emergence	Number of days from host death to first observation of IJ emergence from cadaver
	E	Emergence duration	Total number of days over which IJ emergence from the cadaver is recorded
	I	IJ yield	Total number of IJs emerged per cadaver across the full emergence period
	J	Juvenile cadaver productivity	Percentage of confirmed cadavers from which IJ progeny successfully emerge

The infectivity parameters (TRIP) collectively describe the efficiency and completeness of the host-killing and establishment process under a given thermal condition. Time to death (T) and larval mortality response (R) reflect the speed and magnitude of lethality, which are direct outcomes of IJ penetration success and symbiont efficacy within the host. Infection intensity (I), quantified by direct dissection and enumeration of established IJs within host cadavers, provides a finer-grained measure of infection load independent of mortality alone; a cadaver may be killed while harbouring very few successfully established nematodes, which has downstream consequences for reproductive output. The proportion of larvae producing IJs (P) distinguishes between larvae that die from infection and larvae that die from infection and subsequently support viable nematode reproduction, a distinction that is biologically meaningful and not captured by mortality figures alone (Shapiro-Ilan et al., 2012; Lewis & Clarke, 2012).

The reproductive parameters (LEIJ) describe the temporal dynamics and magnitude of IJ production from confirmed cadavers. Latency to first emergence (L) reflects the speed at which the reproductive cycle completes under a given temperature, with higher temperatures typically accelerating development up to a thermal ceiling (Grewal et al., 1994). Emergence duration (E) captures the temporal spread of IJ release, which has ecological implications for the window of infectivity available to the next host generation. IJ yield (I) is the primary measure of reproductive output and the most direct indicator of population amplification potential per infection event. Juvenile cadaver productivity (J) complements IJ yield by recording the proportion of cadavers that actually release progeny, distinguishing cases where nematodes established successfully within a host but failed to complete their reproductive cycle, a scenario that becomes more common at thermal extremes (Gaugler, 2002; Boemare, 2002).

2.3 Temperature-Response Logic

The utility of the TRIPLE-IJ framework lies not in the measurement of any single parameter in isolation, but in the simultaneous profiling of all eight parameters across a thermal gradient. When plotted together, these parameters produce an integrated performance curve that reveals four ecologically important features of an EPN isolate: its thermal minimum for infectivity, its thermal minimum for reproduction, its optimal thermal range for both phases combined, and its thermal maximum beyond which either or both phases fail. The gap between the thermal range permissive for infectivity and the range permissive for reproduction is of particular diagnostic value, as it identifies temperatures at which an isolate may kill hosts but fail to replenish its field population (Grewal et al., 1994; Hazir et al., 2001).

This approach extends the foundational thermal niche concept established by Grewal et al. (1994), who demonstrated species-level variation in thermal breadth across infection, establishment, and reproduction in twelve EPN species and strains. TRIPLE-IJ builds on this foundation by providing a standardised, isolate-level parameter set that enables systematic comparison across studies, hosts, and geographic origins, rather than the selective reporting of single endpoints that currently dominates the literature (Shapiro-Ilan et al., 2006; Stuart et al., 2006).

2.4 Illustrative Application

To illustrate how the TRIPLE-IJ framework operates in practice, consider two indigenous Nigerian isolates evaluated against third-instar larvae of *Spodoptera frugiperda* across a range of temperatures representing field-realistic conditions in Nigerian maize agroecosystems. An isolate such as *Heterorhabditis bacteriophora* Ib-CRIN68, classified as a cruise forager, may demonstrate high larval mortality (R) and rapid time to death (T) at intermediate temperatures, while showing reduced IJ yield (I) and extended latency to emergence (L) at temperature extremes. A contrasting isolate such as *Steinernema carpocapsae* Ib-ITUC102 may exhibit broader thermal tolerance in the infectivity phase while showing narrower reproductive thermal breadth, producing fewer cadavers with viable progeny (J) at higher temperatures (Okolo et al., 2025). These differences, invisible when mortality alone is reported, become diagnostically clear within the TRIPLE-IJ parameter profile and have direct implications for isolate selection in biocontrol programme design. It should be noted that the isolate-specific examples cited here serve as conceptual

illustrations of framework application, and the reader is directed to the primary experimental manuscripts for full statistical treatment of these findings.

2.5 Advancing Beyond Current Reporting Standards

The EPN literature has long lacked standardisation in how virulence and reproductive performance are reported, with studies varying widely in the parameters measured, the host species used, the temperature conditions tested, and the statistical methods applied (Georgis et al., 2006; Lacey & Georgis, 2012). This variability severely limits cross-study comparability and hampers evidence synthesis for biocontrol programme design. TRIPLE-IJ does not prescribe a single experimental protocol; it prescribes a minimum parameter set. Any researcher using any host system can apply the eight-parameter framework to their own experimental design and produce data that are directly comparable across isolates, species, and geographic contexts. This standardisation function is, in the author's view, among the most practically significant contributions of the framework.

3. The SHIFT Framework

3.1 Conceptual Rationale

The TRIPLE-IJ framework characterises what an EPN isolate does inside a host: how efficiently it kills, how intensively it establishes, and how productively it reproduces under thermal stress. But biocontrol efficacy does not end at cadaver emergence. The infective juveniles that exit the host cadaver must then survive the soil environment, move through it, locate a new host, and initiate a fresh infection cycle before their energy reserves are depleted. It is during this free-living interlude, bounded on one side by cadaver emergence and on the other by successful host penetration, that EPN populations face their greatest attrition in tropical and subtropical field conditions (Glazer, 2002; Maushe et al., 2023).

The SHIFT framework addresses this post-emergence performance dimension. Its full designation, Stress Hurdles and IJ Foraging Traits, reflects the two complementary axes along which ecological fitness is assessed. The first axis, Stress Hurdles, captures the capacity of IJs to survive three major categories of abiotic challenge encountered in field soils: desiccation, hypoxia, and oxidative stress. The second axis, IJ Foraging Traits, captures the host-seeking behaviour and movement ecology of the IJ stage, which determines both the rate of host encounter and the degree of exposure

to each stress hurdle. Together, these axes define the ecological performance envelope of an isolate after reproductive emergence (Lewis et al., 2006; Stuart et al., 2006; Campos-Herrera, 2015).

3.2 The Three Stress Hurdles

Desiccation. Desiccation is widely regarded as the most immediately threatening abiotic stressor for free-living EPN IJs, particularly in the semi-arid and seasonally dry cropping environments characteristic of much of sub-Saharan Africa. IJs lose water across their cuticle at rates that vary significantly across species and isolates, and survival at low water potential differs substantially both between and within EPN genera (Glazer, 2002; Patel et al., 1997; Shapiro-Ilan et al., 2014). The desiccation hurdle is not simply a binary pass-or-fail condition; it operates along a gradient of water potential and exposure duration, and its ecological significance is amplified in surface soils where the greatest density of larval hosts of species such as *S. frugiperda* are typically found during their feeding stages. An isolate that reproduces well within a host but whose progeny IJs cannot persist in dry surface soils has lost a critical functional link in the recycling chain.

Hypoxia. Hypoxia represents a less immediately obvious but ecologically significant constraint, particularly in heavier soils prone to waterlogging following irrigation or rainfall, conditions that characterise much of Nigerian maize-growing terrain during the wet season. IJs at depth in saturated or compacted soils may encounter near-zero dissolved oxygen for extended periods. Grewal et al. (2002) demonstrated significant inter-population variation in hypoxia tolerance among natural populations of *Heterorhabditis bacteriophora*, with survival at approximately zero dissolved oxygen varying meaningfully across isolates of diverse geographical origin, underscoring that hypoxia tolerance is a selectable and ecologically relevant trait rather than a fixed characteristic of a genus or species. IJs of cruise-foraging species are at particular risk, given that their host-seeking strategy requires active movement through deeper soil horizons where oxygen availability is most variable (Maushe et al., 2023; Kaya, 1990).

Oxidative stress. Oxidative stress arises from two principal sources in the context of free-living EPN biology: the external soil environment, where reactive oxygen species are generated by microbial activity and redox-active mineral surfaces; and the internal host immune response, where haemocytes produce reactive oxygen species as a first-line defence against invading IJs. Tolerance to oxidative stress has been shown to correlate strongly and positively with IJ longevity in *H. bacteriophora*, suggesting that oxidative stress resistance is a functional component of overall

persistence capacity rather than an isolated biochemical trait (Grewal et al., 2002; Maushe et al., 2023; Ciche et al., 2006).

3.3 IJ Foraging Traits

The movement ecology of the IJ stage is not uniform across EPN species. Since the early formulation of the foraging strategy paradigm, EPN species have been broadly categorised along a continuum between ambush foragers and cruise foragers, with important implications for both host encounter rates and abiotic stress exposure (Lewis et al., 1995; Campbell & Gaugler, 1993; Griffin, 2012). Ambush foragers, typified by *Steinernema carpocapsae*, remain close to the soil surface and employ nictation behaviour, raising the anterior body into the air to attach to mobile, surface-active hosts. Cruise foragers, typified by *Heterorhabditis bacteriophora* and *Steinernema glaseri*, move actively through the soil matrix and are responsive to volatile and contact host cues at greater distances, making them better suited to locating sedentary or cryptic hosts. A number of species, including *Steinernema feltiae*, exhibit intermediate foraging strategies that combine elements of both approaches (Lewis et al., 2006; Griffin, 2012).

Within the SHIFT framework, foraging strategy is not merely a descriptive label; it is a modulating variable that determines how an isolate's stress hurdle profile translates into field performance. An isolate with strong desiccation tolerance and an ambush foraging strategy is functionally well-matched to dry surface-soil environments where mobile hosts are abundant. An isolate with strong hypoxia tolerance and a cruise foraging strategy is better positioned to exploit hosts in deeper, wetter soil horizons. Mismatches between stress hurdle profile and foraging strategy represent predictable field performance failures that are invisible without explicitly characterising both axes (Stuart et al., 2006; Lewis et al., 2006).

3.4 The SHIFT Performance Profile

When the two axes are combined, SHIFT generates a three-by-two performance profile for any given isolate: survival capacity across three stress hurdles, evaluated in relation to two fundamental foraging strategies. This profile provides a structured description of where an isolate is ecologically strong, where it is vulnerable, and what field deployment conditions it is most likely to succeed in. An isolate that tolerates desiccation and oxidative stress well but is hypoxia-sensitive is a strong candidate for applications in light-textured, well-drained surface soils. An isolate

showing the inverse pattern is better suited to heavier soils or sub-surface pest species. The framework thus functions as a rational basis for isolate-to-environment matching, which currently relies largely on empirical trial and error (Shapiro-Ilan et al., 2006; Lacey & Georgis, 2012).

3.5 Complementarity with TRIPLE-IJ

The relationship between TRIPLE-IJ and SHIFT is sequential and non-redundant. TRIPLE-IJ evaluates the capacity to produce progeny IJs under thermal stress; SHIFT evaluates the capacity of those progeny to survive, move, and infect under field-realistic ecological stress. An isolate must perform adequately on both to function as a self-sustaining biocontrol agent in the field. An isolate that scores strongly on TRIPLE-IJ parameters but fails key SHIFT hurdles will suppress pest populations in the short term through direct infection, but will not recycle, and its biocontrol contribution will diminish rapidly with each generation (Georgis et al., 2006; Campos-Herrera, 2015). Only isolates that perform adequately across both frameworks represent genuinely robust biocontrol candidates for tropical deployment.

4. Integrating TRIPLE-IJ and SHIFT: A Unified Performance Profile

4.1 The Two-Gate Filter

The central argument of this paper is that TRIPLE-IJ and SHIFT are not parallel frameworks that describe different aspects of the same phenomenon; they are sequential gates through which a candidate EPN isolate must pass before it can be considered genuinely robust for field-scale biocontrol deployment. This distinction matters because sequential logic is considerably more demanding than parallel logic. Two frameworks operating in parallel allow a strong score on one to compensate for weakness on the other (Fig. 2). Two gates operating in sequence do not: failure at either gate eliminates the isolate as a self-sustaining biocontrol candidate, regardless of how well it performs at the other (Lacey et al., 2015; Georgis et al., 2006). Gate 1 is TRIPLE-IJ. It asks: under field-relevant thermal conditions, can this isolate infect a host, establish within it, kill it, and produce viable progeny from it? An isolate that clears Gate 1 has demonstrated the capacity to convert host biomass into reproductive output at ecologically realistic temperatures.

Gate 2 is SHIFT. It asks: once progeny IJs emerge from the cadaver, can they survive the desiccation, hypoxia, and oxidative stress conditions they are likely to encounter in the target agroecosystem, and can their foraging strategy bring them into contact with new hosts before their

energy reserves are exhausted? An isolate that clears Gate 2 has demonstrated that its reproductive output translates into ecologically active, host-seeking progeny capable of sustaining a field population beyond the initial application event (Glazer, 2002; Lewis et al., 2006).

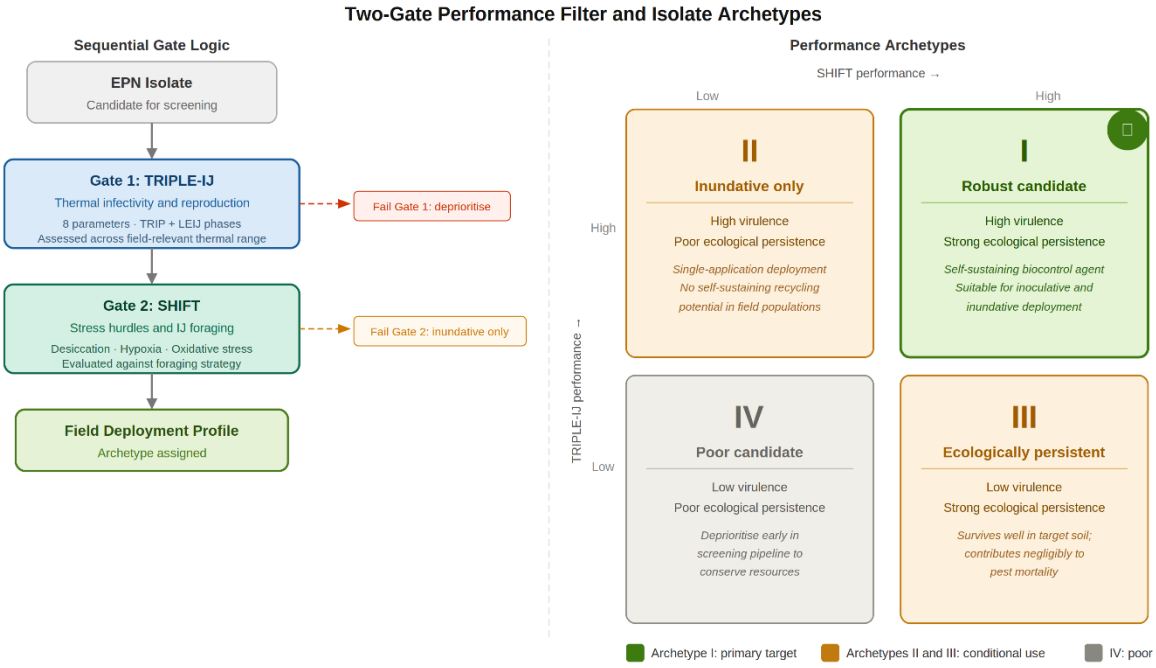

Fig. 2. The two-gate performance filter (left) and associated isolate archetypes (right). The gate flow illustrates the sequential decision logic: EPN isolates must pass Gate 1 (TRIPLE-IJ assessment) before proceeding to Gate 2 (SHIFT assessment), with both gates required for Archetype I classification. The performance matrix shows the four archetypes arranged by TRIPLE-IJ performance (rows) and SHIFT performance (columns).

Only isolates that clear both gates represent candidates for self-sustaining, recycling biocontrol. Isolates that clear Gate 1 but fail Gate 2 provide single-generation suppression, which may be sufficient in some inundative application contexts but cannot be relied upon for population-level pest regulation. Isolates that fail Gate 1 but clear Gate 2 are ecologically persistent but biologically ineffective as biocontrol agents. They occupy soil space and consume resources, but contribute negligibly to pest mortality under the temperature conditions prevailing at the target site.

4.2 Isolate Performance Archetypes

The two-gate logic generates four isolate archetypes that can be used to classify and communicate the performance profile of any characterised isolate. These archetypes are summarised in Table 2 and described below.

Table 2. Four isolate performance archetypes generated by the TRIPLE-IJ and SHIFT two-gate framework.

Archetype	TRIPLE-IJ Performance	SHIFT Performance	Biocontrol Interpretation
I — Robust candidate	High	High	Self-sustaining; suitable for inoculative and inundative deployment
II — Inundative only	High	Low	Effective in single-application programmes; poor recycling potential
III — Ecologically persistent	Low	High	Survives well in target environment; poor virulence under field temperatures
IV — Poor candidate	Low	Low	Neither virulent nor ecologically persistent; unsuitable for deployment

Archetype I isolates are the target of any rigorous EPN selection programme. Their capacity to infect, reproduce, and produce ecologically fit progeny under realistic conditions means that a single application event can initiate a self-amplifying cycle of infection, reproduction, and re-infection within the pest population. In the context of sub-Saharan African smallholder agriculture, where repeated commercial applications may be economically or logistically prohibitive, Archetype I isolates are particularly valuable (Lacey & Georgis, 2012; Abate et al., 2023).

Archetype II isolates are not without value, but they require a different deployment philosophy. They are suited to inundative strategies where very high application rates achieve rapid, short-term pest knockdown, with no expectation of sustained recycling (Shapiro-Ilan et al., 2002; Lacey et al., 2015).

Archetype III isolates represent a form of ecological false positive. Without the TRIPLE-IJ framework, a stress tolerance screening programme might identify such an isolate as a promising

candidate, only for field trials to return disappointing virulence results. SHIFT performance alone is not a proxy for biocontrol potential. Archetype IV isolates should be deprioritised early in the screening pipeline, conserving resources for candidates with greater potential.

4.3 Practical Application in Isolate Screening

In practice, the two-gate framework provides a rational and resource-efficient basis for sequencing isolate screening decisions. TRIPLE-IJ experiments can be conducted first, since virulence bioassays are relatively straightforward and inexpensive. Isolates that fail to demonstrate adequate infectivity and reproductive output across the target thermal range can be deprioritised before the more labour-intensive SHIFT stress tolerance and foraging assays are undertaken (Shapiro-Ilan et al., 2006; Stuart et al., 2006). This sequential screening approach mirrors the logic of the framework itself: Gate 1 is evaluated before Gate 2, and resources are concentrated on the most promising candidates.

For tropical and sub-Saharan African programmes in particular, where indigenous EPN surveys are yielding novel isolates at a rate that outpaces the capacity for full characterisation, this triage function has practical value beyond its conceptual contribution. A common parameter set also enables cross-study comparison, which is currently hampered by the inconsistent reporting practices documented in the introduction (Abate et al., 2023; Lacey & Georgis, 2012).

4.4 Limitations and Caveats

No conceptual framework is without simplifications, and intellectual honesty requires acknowledging them clearly. The two-gate model treats TRIPLE-IJ and SHIFT performance as independent axes, whereas in biological reality they are connected: reproductive output affects the number of IJs available to face ecological stress hurdles, and stress-induced IJ senescence may reduce the virulence of the next infection event. The framework does not model these interactions dynamically. It is a classification tool, not a population model, and it should be understood as such (Stuart et al., 2006; Gaugler, 2002).

Additionally, the three stress hurdles selected for SHIFT represent the most consistently documented constraints on EPN field performance in tropical environments, but they do not constitute an exhaustive list. UV radiation, soil pH, and predation by soil fauna are recognised as additional mortality factors not captured by the current framework (Maushe et al., 2023; Grewal

et al., 2005). Future iterations of the SHIFT component may benefit from incorporating these as the empirical evidence base develops.

5. Implications for EPN Research and Biocontrol in Sub-Saharan Africa

5.1 The Case for Locally Calibrated Frameworks

A persistent challenge in translating EPN research into effective biocontrol programmes in Africa is the uncritical application of performance benchmarks derived from temperate, European, or North American contexts. Commercial EPN products developed and optimised for cool, moist soils at moderate latitudes cannot be assumed to perform equivalently in the thermally variable, seasonally desiccating, and structurally diverse soils of tropical West Africa (Gaugler & Kaya, 1990; Lacey & Georgis, 2012). This is not merely a formulation or logistics problem; it is a fundamental question of ecological fit. An isolate screened and selected under European field conditions carries a TRIPLE-IJ profile and a SHIFT performance envelope calibrated to that environment, not to the agroecological realities of Nigerian maize farms or Kenyan smallholder plots.

The frameworks proposed in this paper offer a direct response to this challenge. Because TRIPLE-IJ and SHIFT are parameter frameworks rather than prescriptive protocols, they can be applied to any isolate under any set of locally relevant temperature and stress conditions. A researcher screening indigenous isolates from the Nigerian Guinea Savanna can apply the full TRIPLE-IJ parameter set across the thermal range actually experienced in maize whorls during the growing season, and can assess SHIFT stress hurdles using soil water potential values and oxygen concentrations representative of local field conditions. The resulting performance profile is therefore ecologically grounded in the target environment, rather than extrapolated from conditions that do not apply (Shapiro-Ilan et al., 2006; Campos-Herrera, 2015).

5.2 The Current Landscape of African EPN Research

Surveys of indigenous EPN diversity across sub-Saharan Africa have accelerated meaningfully over the past two decades. A comprehensive review documented 37 EPN species and 16 species of symbiotic bacteria from surveys spanning Benin, Cameroon, Egypt, Ethiopia, Kenya, Morocco, Nigeria, Rwanda, South Africa, and Tanzania, while noting that in many of these countries, research remains at an early stage and has focused predominantly on laboratory

bioassays with limited ecological characterisation or field validation (Abate et al., 2023). This pattern reflects a structural gap in the research pipeline: isolates are being discovered and their virulence demonstrated under controlled conditions, but the ecological fitness data needed to make rational deployment decisions are largely absent (Tarasco et al., 2023).

TRIPLE-IJ and SHIFT together define precisely the ecological fitness data that are missing. Their adoption as a standard characterisation protocol for newly discovered indigenous isolates would transform the output of survey programmes from a catalogue of virulence demonstrations into an actionable database of deployment-ready performance profiles. This is the most immediate practical contribution the frameworks offer to the African EPN research community (Lacey et al., 2015; Adams et al., 2006).

5.3 A Call for Standardised Parameter Reporting

Beyond their application to isolate screening, the frameworks serve a critical function in addressing the inconsistency of parameter reporting that currently limits evidence synthesis across EPN studies. A meta-analysis of EPN temperature response studies is severely constrained by the fact that different studies report different subsets of endpoints, measured under different conditions, against different hosts, and analysed with different statistical approaches. TRIPLE-IJ does not dictate the experimental conditions; it dictates the minimum parameter set that should be reported. Any researcher working on EPN temperature responses, regardless of their host system, geographic context, or specific research question, can contribute comparable data to the field by reporting all eight TRIPLE-IJ parameters alongside their primary results (Georgis et al., 2006; Lacey & Georgis, 2012).

Similarly, any study characterising EPN stress tolerance can contribute comparable data by framing its findings within the three-hurdle structure of SHIFT and reporting the foraging strategy of the isolate under investigation. The cumulative effect, across studies and laboratories and continents, would be a body of EPN literature capable of supporting the kind of rigorous, quantitative evidence synthesis that biocontrol programme designers in sub-Saharan Africa urgently need (Shapiro-Ilan et al., 2006; Abate et al., 2023).

6. Conclusion

The characterisation of entomopathogenic nematodes for biological control has long generated rich data within individual studies while yielding a body of literature that resists meaningful synthesis across them. Parameters are selected inconsistently, endpoints are reported in isolation, and the ecological fitness of IJs beyond the host cadaver is rarely connected to the virulence data that precede it. The result is a research landscape in which isolates are frequently described but rarely understood in their full performance dimension (Georgis et al., 2006; Lacey & Georgis, 2012).

The TRIPLE-IJ and SHIFT frameworks proposed here represent an attempt to address this structural limitation through a conceptually coherent, parameter-standardised approach to EPN characterisation. TRIPLE-IJ provides a unified, eight-parameter lens through which the dual effects of temperature on infectivity and reproduction can be captured simultaneously and comparably across isolates, hosts, and experimental contexts. SHIFT extends this characterisation into the post-emergence ecological domain, connecting stress tolerance to foraging strategy and thereby to the conditions under which reproduced IJs can actually function in field soils. Together, the frameworks constitute a two-gate performance filter whose adoption would strengthen both isolate screening decisions and the cumulative evidence base on which those decisions depend.

The frameworks were developed from empirical work on indigenous Nigerian EPN isolates, but their logic is not geographically bounded. Any researcher characterising any EPN isolate against any host, in any agroecological context, can apply both frameworks to their own data. That generalisability is the measure of a useful conceptual contribution. The field now has the vocabulary; the next step is to use it consistently.

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