Indian Journal of Entomology Online published Ref. No. e23355 DoI. No.: 10.55446/IJE.2023.1355

PROTOCOL FOR TEMPERATURE TOXICITY INVESTIGATION ON WHITEFLY BEMISIA TABACI (GENNADIUS)

Nikhil R M[1], Anil Kumar S T[1], Subramanian S[1] and Mahapatro G K[1]*

[1]Division of Entomology, ICAR- Indian Agricultural Research Institute, New Delhi 110012, India
*Email: gagan_gk@rediffmail.com (corresponding author): ORCID ID 0000-0002-9235-1814

ABSTRACT

A cost-effective laboratory protocol was perfected for investigating temperature toxicity against whitefly *Bemisia tabaci* (Genn.) on tomato. Understanding temperature toxicity relationship will aid in strategization of pest management. The experiments conducted to develop the current protocol used *B. tabaci* Asia II-1 as test insect. Essentially this protocol consist of three steps which are: (1) Temperature incubation of test insects: includes three hour starvation period and temperature treatment of *B. tabaci*; (2) Preparatory steps of leaf-dip bioassay: suggests use of agar cube method (reduces agar usage by 70%) to maintain green and turgid test leaves during post-exposure period of bioassay; (3) Release of temperature treated insects and mortality counting: test insect release, securing petri plates, recording observations, etc. all are deliberated in detail. This protocol facilitates economical, convenient and easy-to-handle experimentation to study temperature toxicity relationship in *B. tabaci*.

Key words: Agar cube method, Insecticide Resistance Action committee (IRAC), leaf-dip bioassay, temperature toxicity studies, tomato

Whitefly *Bemisia tabaci* (Gennadius) is a homopteran insect pest of paramount significance (Horowitz et al., 2020; Li et al., 2021). This polyphagous phloem feeder is well known for its pestilence to a vast array of cultivated crops and cause yield losses which are accounted for millions of dollars (Brown, 1994; Oliveira et al., 2001). Currently, the main control strategy in the field to manage this pest is chemical management (Naveen et al., 2017) and a set of insecticides are recommended for specific crops by Central Insecticides Board and Registration Committee (CIBRC, 2023) in India. Adequate studies were done on the bioefficacy of insecticides (Elbert and Nauen, 2000; Naveen et al., 2017; Satar et al., 2018; Kelageri et al., 2022; Rajna et al., 2022) on *B. tabaci* but primarily for insecticidal screening, with limited thrust on temperature-toxicity aspects. Several biotic and abiotic factors cause variation in the bioefficacy of insecticides; among them, the temperature is the most important one. Often this has profound effects on the bioefficacy of insecticides, and these temperature-dependent variations could be due to changes in spray-coverage (Nageshkumar et al., 2021), change in insect behaviour (Shirani-Bidabadi et al., 2022) and altered insecticide toxicity (Scott, 1995). Notably, there is a gap in understanding how this test insect reacts to different insecticides at different temperature regimes. Obviously this is due to high mortality in experimental setups, especially when the experimentation involves two cardinal variables, i.e. temperature and toxicity, the small size and difficulties in handling *B. tabaci*.

Negligible studies on the temperature-toxicity relationship of *B. tabaci* and insecticides are available. Temperature-toxicity investigation is primarily of two types (based on pre- and post-temperature). Post temperature-toxicity study simultaneously using temperature post-treatment exposes both the test insects and test-diet (host leaf) under temperature and toxicity treatment in tandem (Li et al., 2020; Khan, 2021). In caterpillar insect pests, conducting post exposure treatments is convenient. But drying and denaturation of tomato (*Solanum lycopersicum* Mill) leaves at elevated temperature during long post-exposure hours render the leaves unsuitable for normal feeding of the *B. tabaci* in temperature post-treatments. This practical limitation encourages one to adopt temperature pre-treatment toxicity studies, wherein temperature treatment was given to the test insect prior to insecticide treatment. This gap in temperature-toxicity investigation for *B. tabaci* is due to the unavailability of appropriate practical protocol(s) for studying the temperature-toxicity relationships. Hence, the prime objective of this study is to perfect a protocol for temperature-toxicity study in *B. tabaci* using currently available knowledge (Cui et al., 2008; Mahadav et al., 2009; IRAC, 2016; Naveen et al., 2017; Guo et al., 2018) and some experimentally derived information.

MATERIALS AND METHODS

Reagents and equipment used in the study and proposed protocol are agar agarose, aluminium foil, tomato (*Solanum lycopersicum* Mill.) leaves, double distilled water, insecticides, CO_2 cylinder with pressure regulator, Petri plates (90x15 mm), needle, candles, handheld mouth aspirator (length 16 cm, diameter 3.5 cm), black cloth, white cloth, forceps, hand gloves, hot plate, beakers, measuring cylinder, micropipette (100-1000 µl) with tips, graduated pipette (5 ml), BOD incubator, blotting paper, parafilm® m tape and refrigerator. *B. tabaci* population was collected from unsprayed tomato field (28° 37' 41" N, 77° 09' 36" E) in IARI, Pusa, New Delhi and reared on potted (diameter 15 cm) tomato plants in a protected and controlled environment under temperature 27± 1°C and 65± 5% RH and photoperiod 14:10-L:D at Insect Proof Climate Control Chamber, Division of Entomology, ICAR-IARI, New Delhi (during 2018-22). Genetic group identification of *B. tabaci* was done in accordance to the methodology described by Ramesh et al. (2022). DNA was isolated from single adult *B. tabaci* by using DNeasy® Blood and Tissue kit (Qiagen). From the extracted DNA, a part of mitochondrial cytochrome oxidase I (mtCOI) was amplified using universal primers C1-J-2195 (Forward primer; 5'-TTGATTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (Reverse primer; 5'-TCCAATGCACTAATCTGCCATATTA-3') using polymerase chain reaction (Simon et al., 1994). The amplified product was purified and subjected to Sanger sequencing (Green genome, New Delhi). The sequence was aligned using Clustal W using software BioEdit v7.2.5 then analysed for phylogeny using MEGA X (Kimura, 1980; Kumar et al., 2018). The generated mtCOI sequence was submitted to NCBI GenBank to get accession number.

Agar is used to keep the leaves green and turgid during the post-exposure period by inserting tomato leaf petiole into it. The effectiveness of this in leaf dip bioassay (IRAC, 2016) was compared for the following three agar methods viz., agar full plate method; 20 ml agar solution (1%) poured evenly at the base of a Petri plate (3-4 mm thickness) and allowed to solidify (IRAC, 2016), using agar slant method, were 10 ml agar solution (2%) is poured into Petri plate which is kept at an angle and allowed to solidify (Naveen et al., 2017) and using agar cube method, evaluated by us, where 2% agar solution is poured evenly (1-1.2 cm thickness) in a separate container and after solidification is cut into cubes (1x1x1 cm to 1.2x1.2x1.2 cm) using a sharp blade and onto this agar cube leaf petiole is inserted and placed in the Petri plate. These treatments are compared with respect to control mortality (using double distilled water for leaf dip) at 27± 1°C and 65± 5% RH at 24, 48 and 72 hr. Treatments are replicated 10 times. These three methods for agar usage will also reflect in the quantity of agar required for the experiment and thus the cost for experiment might change. This is analysed by calculating the average quantity of agar needed for preparing 1000 Petri plates and deriving the cost of agar by current market prices.

Test insects were subjected to control temperature-incubation inside the handheld aspirator (length 16 cm, diameter 3.5 cm) itself to minimize handling damages to the insects. The aspirator is modified in two ways, using black cloth and white cloth covering both ends of the aspirator, under which the glass wall of aspirator and slanting cork plug form a crevice, into which *B. tabaci* tend to crawl up. An unmodified aspirator with no cloth covering at both ends was used as the third treatment (control). Hundred adult insects per aspirator were collected and then kept one end facing a light source, so insects positive phototropic nature combined with its tendency to move into the crevice make a perfectly unfavourable situation for incubation. Experiments were conducted at temperature 27± 1°C and 65± 5% RH and treatments are replicated 10 times. Mortalities were counted at 0.5 and 3.0 h. Temperature stress on *B. tabaci* was investigated according to the methodology described by Guo et al. (2018) with slight modification. Hundred adult insects were collected in modified handheld mouth aspirator (with black cloths covering both ends) and subjected to different temperature treatments (23, 27, 31, 35 and 39°C) for specified time periods (15 min, 45 min, 1.5 h and 3 h) in BOD incubator at 65± 5% RH. The mortality observations were taken after incubation, each treatment was replicated thrice. Data obtained by the experiments were analysed in SPSS (version 16.0). Mean values compared by ANOVA and statistical significance at P=0.05 was calculated using Tukey test.

RESULTS AND DISCUSSION

The accession number allotted by GenBank was OQ402684. This mtCOI sequence (820 bp) was used to construct phylogenetic tree of *B. tabaci* using maximum likelihood approach and it was confirmed as Asia II-1 genetic group (Fig. 1). The prevalence of genetic group Asia II-1 is well recorded in North Indian region

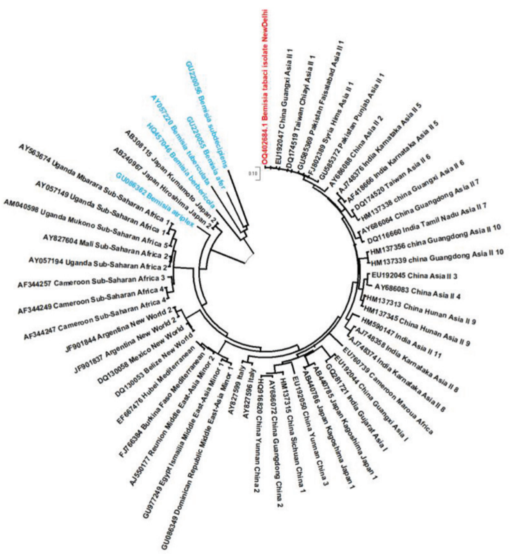

Fig. 1. Phylogenetic tree of mtCOI genetic groups of *Bemisia tabaci* constructed using maximum likelihood approach in MEGA X, showing position of selected population (accession no.- OQ402684 New Delhi India: Red colour text). Other cryptic species are selected as outgroups (Blue colour), all the sequences other than selected one were obtained from GenBank.

(Ellango et al., 2015; Ramesh et al., 2022). The same genetic group was also reported by Naveen et al. (2017) in New Delhi in cotton. As different genetic groups of *B. tabaci* shows differential temperature tolerance (Mahadav et al., 2009) it is important to identify the genetic group of *B. tabaci* during temperature toxicity investigations. The results of control mortality in leaf-dip experiments using different agar methods shows that the agar cube method (2C in Fig. 2) has a control mortality of 1.13±0.18, 2.36± 0.19 and 5.45± 0.46% at 24, 48 and 72 hr, respectively. While, agar slant method (Naveen et al., 2017) recored control mortalities of 1.38± 0.24, 2.49± 0.34 and 6.18± 0.52% at 24, 48 and 72 hr, respectively and agar plate method (IRAC, 2016) exhibited control mortalities of 1.27± 0.21, 2.50± 0.26 and 6.12± 0.48% at 24, 48 and 72 hr, respectively. The control mortality obtained in agar cube method are on par with the other two methods and there is no significant difference among these three methods with respect to control mortality.

During the cost comparison, it was evident that the agar cube method requires a very small quantity of agar/ 1000 petri plates, i.e. 60 g, at the current market price of agar, i.e. approximately 8000 rupees/ kg. This will cost 480 rupees/ 1000 petri plates. Whereas, the other two methods require 200 g of agar for the preparation of 1000 petri plates, which will cost 1600 rupees/ 1000 petri plates. Thus, by adopting agar cube method, one saves substantially, as it reduces agar usage by 70% and cuts agar costs by 70%, given that agar is a costly recurring expenditure. All these control mortalities in leaf-dip method of bioassay using agar methods have acceptable range of control mortality by Insecticide Resistance Action Committee (IRAC) standards i.e. control mortality in a bioassay exceeds over 20% experiment is inferior and needs to be repeated. Ideally control mortality should not exceed 10-15% (IRAC, 2016). Moreover agar cube method allows minimal use of the experimental resource (agar) and thus economical. This also gives added advantage of storability and ease of usage i.e., the agar cubes can be prepared before the experiment, stored in a clean container covered with aluminium foil in a normal refrigerator for up to seven days and is ready to use whenever needed. Thus agar cube method can be recommended in the leaf dip method of bioassay using petiolate leaves making it more convenient, cheaper and easier.

When incubation mortality within the aspirator was compared, an unmodified aspirator with no cloth covering both ends resulted in insects crawling into the crevice created by the glass wall of the aspirator and slanting cork plug, which resulted in 18.14± 1.58 and 24.34± 1.76% mortality in 0.5 and 3 hr, respectively. While aspirator modified with black cloth covered at both ends recorded having least mortality i.e. 0.00 and 1.75± 0.31% mortality in 0.5 and 3 hr and white cloth covered aspirator was on par with black cloth covered aspirated with 0.00 and 2.78± 0.35% mortality in 0.5 and 3 hr. Temperature incubation of *B. tabaci* was carried out in the aspirator itself. This was to reduce the handling damage as the test insect is very small and delicate in nature. But the insect is having a peculiar behaviour that it tends to crawl up into the crevice created by the glass wall and slanting cork plug and as a result, gets killed in that tight confined space. During incubation this will result in the loss of test insects. So modifying the aspirator with black/ white cloth helps to reduce the mortality as insects prefer to rest at the middle of the aspirator away from the two dark ends. This modified aspirator using black/ white cloth can be used to incubate *B. tabaci* in a BOD incubator at temperature range of 23-35°C and 65± 5% RH.

Under temperature stress study it was evident that *B. tabaci* adults were able to survive up to 35°C without any significant mortality. At 39°C it started to exhibit significant decline in survivability and longer exposure periods resulted in higher mortality rates. Similar work was done by Cui et al. (2008) who reported that 1 hr temperature treatment of *B. tabaci* up to 37°C does not show any significant mortalities

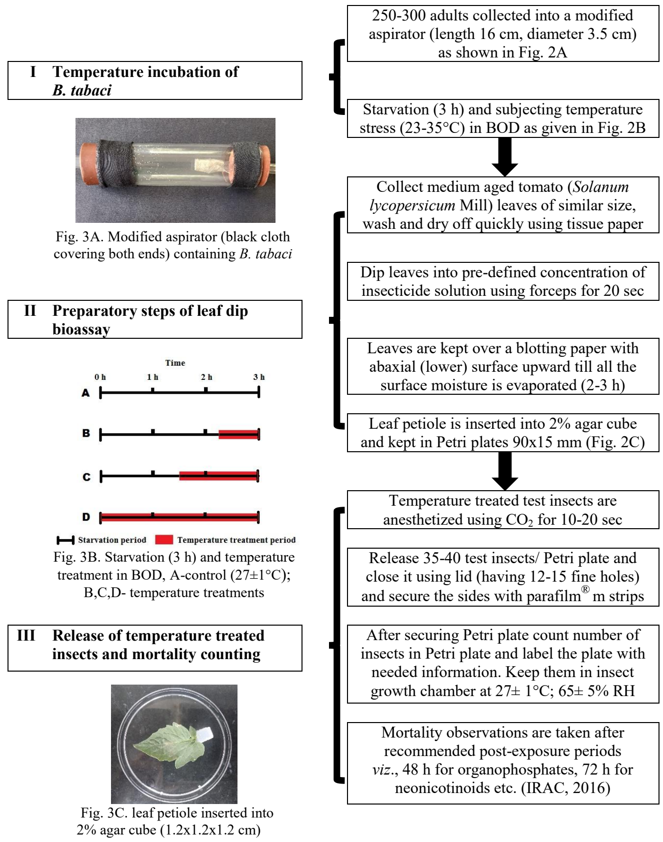

Fig. 2. Protocol for temperature toxicity study on *B. tabaci* on tomato

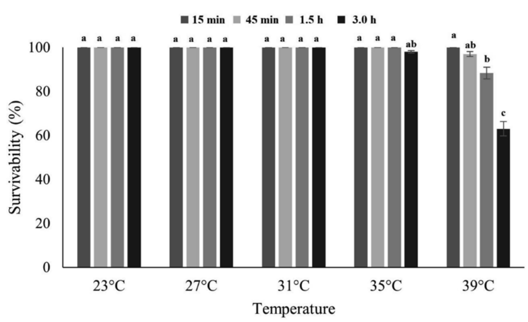

Fig. 3. Survival of *Bemisia tabaci* in response to temperature stress (Mean and SEM are indicated, statistical significance at p=0.05 calculated by Tukey test)

for both female and males at the adult stage. Whereas, when subjected to 37°C for an extended period of 3.5 hr, both B and Q *B. tabaci* biotypes exhibit significant mortalities (Mahadav et al., 2009). Guo et al. (2018) used the temperature treatment procedure given by Cui et al. (2008) and reported that at 31 and 35°C *B. tabaci* shows no significant mortality. That is why upper limit of temperature toxicity study as 35°C has been fixed. The present laboratory temperature stress study has combined the pre-existing knowledge of *B. tabaci* survival under various high temperature ranges, and hence temperature range for temperature toxicity investigation was fixed between 23-35°C. This temperature range is optimum for fixing temperature regimes for temperature toxicity investigations. This adaptive range assures the survival of the test insects for the required experimentation. The temperature toxicity protocol developed for *B. tabaci* is described as a flowchart in Fig. 2.

The protocol will help investigate the temperature toxicity relationships in *B. tabaci* and make the experiment economic, convenient and practical. The problems arising in post-temperature post-treatment studies, such as drying and denaturation of succulent tomato leaves at high temperature treatment, during long bioassay cause hindrances in the normal feeding of the *B. tabaci*. This practical limitation can be mitigated following this protocol. The three-step protocol has been deliberated in detail in the form of a flowchart and provided with some illustrations/images for more clarity for the readers/researchers (Fig. 2). This protocol is applicable for various insecticide classes, viz., neonicotinoids, TRPV channel modulators, flonicamid, pyrethroids, organophosphates, and other *B. tabaci* adulticides.

ACKNOWLEDGEMENTS

This study is part of the doctoral research work of the first author under the chairmanship of Dr. Mahapatro G K. The authors are grateful to the authorities of ICAR-IARI, New Delhi for providing the essential resources and facilities to support the PhD thesis work.

FINANCIAL SUPPORT

The first author is grateful to the authorities of ICAR-IARI, New Delhi for providing financial support in the form of IARI fellowship during the tenure of his doctoral degree.

AUTHOR CONTRIBUTION STATEMENT

NRM and MGK conceived and designed research, NRM conducted experiments, NRM and AKST analysed data, SS and MGK provided research facilities, NRM wrote manuscript and MGK corrected manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Brown J K. 1994. The status of *Bemisia tabaci* (Genn.) as a pest and vector in world agro ecosystems. FAO Plant Protection Bulletin 42: 3-32.

CIBRC (Central Insecticides Board and Registration Committee). 2023. MUP: Major uses of pesticides. Ministry of Agriculture & Farmers Welfare, Government of India; http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides

Cui X, Wan F, Xie M, Liu T. 2008. Effects of heat shock on survival and reproduction of two whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B. Journal of Insect Science 8(1): 1-10.

Elbert A, Nauen R. 2000. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. Pest Management Science 56(1): 60-64.

Ellango R, Singh S T, Rana V S, Gayatri Priya N, Raina H, Chaubey R, Naveen N C, Mahmood R, Ramamurthy V V, Asokan R, Rajagopal R. 2015. Distribution of *Bemisia tabaci* genetic groups in India. Environmental Entomology 44(4): 1258-1264.

Guo L, Su M, Liang P, Li S, Chu D. 2018. Effects of high temperature on insecticide tolerance in whitefly *Bemisia tabaci* (Gennadius) Q biotype. Pesticide Biochemistry and Physiology 150: 97-104.

Horowitz A R, Ghanim M, Roditakis E, Nauen R, Ishaaya I. 2020. Insecticide resistance and its management in *Bemisia tabaci* species. Journal of Pest Science 93: 893-910.

IRAC (Insecticide Resistance Action Committee). 2016. IRAC susceptibility test method 015. IRAC. https://irac-online.org/methods/ trialeurodes-vaporariorum-bemisia-tabaci-adult/

Kelageri S S, Mahapatro G K, Subramanian S, Srivastava C, Rajna S. 2022. Relative susceptibility of life stages of cotton whitefly *Bemisia tabaci* (Genn.) to pyriproxyfen. Indian Journal of Entomology e21188: 1-5. DoI.: 10.55446/IJE.2021.370

Khan H A A. 2021. Posttreatment temperature influences toxicity of insect growth regulators in *Musca domestica*. Parasitology Research 120(2): 435-441.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111-120.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547-1549.

Li Y, Dou Y N, An J, Tu X, Lv H, Pan W, Dang Z, Gao Z. 2020. Temperature-dependent variations in toxicity of diamide insecticides against three lepidopteran insects. Ecotoxicology 29: 607-612.

Li Y, Mbata G N, Punnuri S, Simmons A M, Shapiro-Ilan D I. 2021. *Bemisia tabaci* on vegetables in the southern United States: incidence, impact, and management. Insects 12(3): 198.

Mahadav A, Kontsedalov S, Czosnek H, Ghanim M. 2009. Thermotolerance and gene expression following heat stress in the whitefly *Bemisia tabaci* B and Q biotypes. Insect Biochemistry and Molecular Biology 39(10): 668-676.

Nageshkumar T, Anantachar M, Veerangouda M, Prakash K V, Nadagouda S. 2021. Comparative evaluation of some sprayers in control of leaf hoppers and aphids of cotton crop. Journal of Entomological Research 45: 971-977.

Naveen N C, Chaubey R, Kumar D, Rebijith K B, Rajagopal R, Subrahmanyam B, Subramanian S. 2017. Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I, Asia-II-1 and Asia-II-7 on the Indian subcontinent. Scientific Reports 7(1): 1-15.

Oliveira M R V, Henneberry T E, Anderson P. 2001. History, current status, and collaborative research projects for *Bemisia tabaci*. Crop Protection 20(9): 709-723.

Rajna S, Mahapatro G K, Subramanian S, Chander S, Kelageri S. 2022. Susceptibility of imidacloprid resistant whitefly *Bemisia tabaci* (Gennadius) to cyantraniliprole. Indian Journal of Entomology 84(3): 663-666.

Ramesh K B, Mahendra C, Kelageri S S, Rajna S, Subramanian S. 2022. Distribution and mitotype diversity of *Bemisia tabaci*. Indian Journal of Entomology e22710: 1-5.

Satar G, Ulusoy M R, Nauen R, Dong K. 2018. Neonicotinoid insecticide resistance among populations of *Bemisia tabaci* in the Mediterranean region of Turkey. Bulletin of Insectology 71(2): 171-177.

Scott J G. 1995. Effects of temperature on insecticide toxicity. pp. 111-135. Roe R M, Kuhr R J (eds.). Reviews in Pesticide Toxicology Vol 3. Toxicology Communications, Raleigh, NC, USA.

Shirani-Bidabadi L, Oshaghi M A, Enayati A A, Akhavan A A, Zahraei-Ramazani A R, Yaghoobi-Ershadi M R, Rassi Y, Aghaei-Afshar A, Koosha M, Arandian M H, Ghanei M. 2022. Molecular and biochemical detection of insecticide resistance in the leishmania vector, *Phlebotomus papatasi* (Diptera: Psychodidae) to dichloro diphenyl trichloroethane and pyrethroids, in central Iran. Journal of Medical Entomology 59(4): 1347-1354.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651-701.

(Manuscript Received: June, 2023; Revised: July, 2023;
Accepted: August, 2023; Online Published: September, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23355